POSSIBLE ATTENUATION OF DEVELOPMENTAL EFFECTS OF ADOLESCENT EXPOSURE TO METHAMPHETAMINE BY CONCURRENT TREATMENT WITH NALTREXONE

A thesis submitted in fulfilment of the requirements for the

Degree of Master of Science in Psychology

At the University of Canterbury

by

Amy MacLachlan

University of Canterbury

2020

Acknowledgements

Firstly, I would like to acknowledge the support of my primary supervisor, Professor Rob Hughes. Throughout the duration of my thesis you gave input and advice that allowed the completion of my thesis to be possible. Thank you for your patience in both answering my questions and teaching me what I needed to know.

Thank you also to my secondary supervisor, Professor Randolph Grace, for lending your wisdom and knowledge of statistical analysis and writing.

To Neroli and Silvana I thank for all the support and guidance that you provided to me while I worked in the laboratory.

I would like to sincerely thank my parents Nic and Warwick, and my brother Campbell. You have each uniquely provided me with support and love to get me through this past year. To you, Campbell, I thank for your academic wisdom and providing an unconditional source of knowledge and support whenever I was lost. To my parents, I thank for your concern, your prayers, and your constant encouragement. Thank you, mum, for painstakingly reading through my thesis and giving advice on my writing.

To my friends, I thank for your encouragement. Liz and Nat, you have both been unwavering motivational support pillars that have gotten me through not only this past year, but my entire academic career. Thank you for the uplifting conversations, and for the constant reminder of the light at the end of the tunnel.

Dear David. Thank you for putting up with me throughout this journey. Thank you also for your prayers, your caring support and your optimistic belief in my ability to get this done.

Most of all, thank you God- the greatest Master of all psychological sciences.

Table of Contents

Abbreviations

MA	Methamphetamine
WM	Working memory
PND	Post-natal day
LA	Locomotor activity
PFC	Pre-frontal cortex
DA	Dopamine
GABA	Gamma aminobutyric acid
ACh	Acetylcholine
VTA	Ventral tegmental area
NAc	Nucleus accumbens
EA	Late adolescence/early adulthood

List of Tables

List of Figures

Abstract

Methamphetamine (MA) is an illicit psychostimulant drug with high abuse liability and addictive potential. The predominance of MA research focuses on its effects in adulthood however its use is typically initiated in adolescence. This begs the question: do the neuropsychological and behavioural effects of MA differ between adulthood and adolescence. Of particular interest are differences pertaining to MA-induced anxiety and (spatial) working memory (WM) deficits. Due to the current paucity of research there is insufficient insight into the differential long-term effects of adolescent versus adult MA-exposure. However, there remains the potential of adolescent vulnerability, warranting further research into adolescent effects. While specific investigations are lacking, the existing evidence indirectly suggests a mechanism by which the adolescent brain is more vulnerable to enduring drug-induced behavioural and psychological changes. Moreover, MA dependence lacks effacious treatment modalities and there are no approved pharmacotherapeutic agents. To that end, the efficacy of the novel agent naltrexone was investigated. The present research aimed to determine the effects of naltrexone in MA-exposed adolescent rats, on the development of heightened anxiety and memory impairments in early or later adulthood. Possible sex differences in responsiveness to the treatments were also assessed. Rats were administered drug treatment of once daily saline or naltrexone (10.0mg/kg) 15 minutes prior to another dose of saline or MA (1.0 or 2.0 mg/kg) from post-natal days (PND) 41-50. Each rat underwent subsequent behavioural testing in each of three testing apparatuses: the light/dark box, the zero maze, and the y-maze. These tests incorporated certain measures to determine anxiety and spatial WM behaviour at PND60 and PND100. Overall, MA did not clearly or consistently worsen, nor did naltrexone clearly or consistently attenuate, anxiety or WM performance behaviour in early or later adulthood. Females had lower anxiety than males, indicating that sex independently impacts anxiety-related behaviour in rats. I concluded that MA did not worsen anxiety and WM performance, and that naltrexone did not attenuate these effects in later adulthood. Naltrexone was expected to attenuate MA's effects but was not expected to produce anxiolytic or memory enhancing action alone.

Naltrexone may not have demonstrated any overall attenuating effect because MA did not demonstrate any overall anxiogenic or reduced WM effect. Indeed, the lack of anxiogenic and reduced WM effects of MA could plausibly be explained by testing flaws and/or the confounding effect of general activity. The outcomes of the present investigation are not sufficient to draw conclusions regarding the long-term effects of adolescent-MA or naltrexone use in rats, and further research is necessary to elucidate these effects. Future research should also continue to investigate these effects for both females and males due to the clear demonstrated difference in their behaviour in the present research.

Introduction

Methamphetamine (MA) is a highly addictive psychostimulant (Luikinga, Kim, & Perry, 2018) of the phenethylamine and amphetamine class of psychoactive drugs (Courtney & Ray, 2014). MA dependence is a current worldwide public health problem, associated with serious deleterious outcomes for physical and mental health (Petit, Karila, Chalmin, & Lejoyeux, 2012). There remains a relative paucity of research in both humans and animals (Buck & Siegel, 2015) of its effects, particularly regarding the long-term effects from exposure during the period of adolescence (Luikinga et al., 2018). The limited research on record has mostly focused on the effects of MA use in adults (Buck & Siegel, 2015). Yet most drug use, including MA, tends to start at a younger age, particularly during adolescence (Luikinga et al., 2018). Additionally, outcomes may differ based on the age at which MA use begins. Therefore, it is vitally important to investigate whether differential effects exist, to determine if adolescent-based treatment targets are necessary.

Developmental Effects of MA

The term 'adolescence' represents the period when childhood transitions into adulthood, involving various developmental changes (Spear, 2000). For this reason, research has suggested that the behavioural and neuropsychological effects of MA in adolescents may differ from those for adults (Luikinga et al., 2018). For example, addiction risk is higher for MA-exposed adolescents due to their greater sensitivity to the reinforcing and rewarding properties of drugs of abuse in general (Schramm-Sapyta, Walker, Caster, Levin, & Kuhn, 2009; Volkow & Morales, 2015). Anxiety and memory effects may also differ. Anxiety is a common symptom accompanying MA use (Cruickshank & Dyer, 2009) and withdrawal (Mancino, Gentry, Feldman, Mendelson, & Oliveto, 2011), and research is not conclusive about how to manage anxiety in MA users (Hellem, 2016). Cognitive dysfunction (which involves working memory (WM) function) is a feature of chronic psychostimulant abuse (Sherrill, Stanis, & Gulley, 2013). Animal research models have also

demonstrated that repeated MA use induces spatial WM impairments (Lee, Kim, Lee, & Jang, 2011; Nagai et al., 2007). Our study investigated a specific period of adolescence in a rodent model, namely post-natal days (PND) 41-50. Accordingly, the present literature review focuses on existing research investigating this period.

Spatial WM Effects of MA

From the limited research available, results have indicated that long-term adolescent MA-induced WM (including spatial WM) effects occur during some stages of adolescence and not others. Multiple daily administrations of neurotoxic (6.25 mg/kg) doses of MA between PND41-50 (periadolescence) have produced spatial learning deficits 30 days after treatment in later adulthood as shown in the Morris water maze. Administration of smaller doses, and at other developmental stages (PND21-30, 30-40, and 51-60) produced no MA-induced effect (Vorhees et al., 2005). Research by Ye, Pozos, Phillips, and Izquierdo (2014) also found that daily MA (starting with 0.3mg/kg increasing by 0.3mg/kg per day thereafter) between PND41-50 produced long-term generalised learning deficits in adulthood relative to controls (saline). Neither Vorhees et al. (2005) or Ye et al. (2014) used adults as a comparison in their investigations. However, they both respectively illustrated a long-term effect of neurotoxic and non-neurotoxic MA exposure on memory function during periadolescence.

Sherrill et al. (2013) exposed rats to repeated amphetamine (3mg/kg 10 total injections, once every second day) during adolescence (PND37-P55). These rats experienced greater general WM deficits in later adulthood relative to control rats and rats exposed to repeated amphetamine during adulthood (PND98-116) (Sherrill et al., 2013). This indicates that adolescence is probably a period of heightened vulnerability to long-term MA-induced WM deficits relative to adults. Whilst Sherrill et al. (2013) investigated PND50-51 as per Vorhees et al. (2005), they did not do so exclusively, so it is difficult to determine what part of the PND37-PND55 age period was causing the effect.

However, research findings from White, Minamoto, Odell, Mayhorn, and White (2009) shed more light on MA-induced effects in this PND50 plus age range. Consistent with Vorhees et al.

(2005) rats administered daily MA (9mg/kg) during PND50-51 had no impaired spatial discrimination acquisition during adulthood (White et al., 2009). This indicates that the effect reported by Sherrill et al. (2013) was less likely to have arisen from MA administration around PND50 and above. It also further suggests that peri-adolescence is a period of vulnerability to the long-term developmental effects of MA-exposure on WM function. However, this effect found by White et al. (2009) may have also been due to their shorter period of MA administration.

Other research indicates that vulnerability to MA-induced effects may extend outside the peri-adolescent period. North et al. (2013) demonstrated that repeated 14 once-daily 24mg/kg MA administrations in adolescent rats aged between 4-5 weeks (PND28-35) led to a long-lasting spatial WM deficit after 7, 14, and 21 days of MA abstinence relative to controls. These findings did not suggest a long-term memory deficit effect in later adulthood. However, it did demonstrate that adolescent-MA use produces a lasting deficit in spatial WM after a long period of abstinence in later adolescence/early adulthood (EA). With respect to a long-term MA-induced WM deficit, this is consistent with findings of all of the described research, except for those of White et al. (2009).

Irrespective of North et al. (2013) findings of adolescent vulnerability to MA outside of periadolescence, these results collectively suggest that a critical time-window exists during this developmental period of adolescence. During this time, MA may have adverse long-term effects on (spatial) WM. However, more research is needed to fully elucidate both these effects, and when during adolescence lies the greatest period of vulnerability.

Anxiety Effects of MA

There is even less research investigating possible age differences in the effects of MA on anxiety. Of this literature, research tends to be focused on acute effects, particularly with respect to locomotor activity (LA). Lower levels of LA are often associated with heightened anxiety (Archer, 1973; Belzung, 1999). Using an open field test, Struntz and Siegel (2018) found no difference between adult (PND109 or 109) or adolescent (PND42 or 43) acute 4mg/kg MA exposure on immediate increases in anxiety, including locomotor behaviour, using an open field test.

Additionally, an increase in locomotor behaviour indicates lower anxiety. However, adolescence is characterised by increased LA (Wahlstrom, Collins, White, & Luciana, 2010), which may explain research results of acute increases in locomotor behaviour during adolescence. MA may also increase locomotor behaviour irrespective of anxiety levels.

In other research, acute administration of 0.125 or 0.5 mg/kg MA induced increased locomotor behaviour, which was larger for adults (PND66-70) than for adolescents (PND34-35). LA was consistent over the 5 daily MA administrations for each group (Zakharova, Leoni, Kichko, & Izenwasser, 2009). Adolescence being 'characterised' by increased LA (Wahlstrom et al., 2010) is not consistent with the finding that adults had a larger increase in LA. Again, an increase in locomotor behaviour implies reduced anxiety. This discrepancy may be explained by the measure of locomotion not being within the context of an anxiety-based test (Zakharova et al., 2009).

Sherrill et al. (2013) administered 10 3mg/kg injections of amphetamine, once every second day over 19 total days. Consistent with the aforementioned findings, both adolescent (PND37-55) and adult-exposed rats showed significant increases in ambulation and stereotypy after the first and tenth days of amphetamine administration. By the tenth day, and in the long term (post MA-treatment), adult-exposed rats showed a significant decrease in ambulation and greater stereotyped behaviour than adolescent-exposed animals (Sherrill et al., 2013). Activity was measured within the context of a pre-frontal cortex (PFC)-sensitive WM task. This task is not specifically used to measure anxiety. Zombeck, Gupta, and Rhodes (2009) reported that 1,2, or 4mg/kg MA exposure during adolescence (PND30-35) led to significantly lower acute increases in locomotion (recorded via home cage tracking) than exposure during adulthood. Again, the authors did not investigate locomotion using a test known to measure anxiety and were investigating locomotor stimulation as opposed to anxiety.

Therefore, due to not adopting acknowledged anxiety-based measures, LA level recorded by Zakharova et al. (2009), Sherrill et al. (2013) and Zombeck et al. (2009) could have been reflecting something other than anxiety. The results of these researchers may have merely been due to the motor activity increases induced by MA and other psychostimulants (Asser & Taba, 2015).

The research described above generally involves the acute effects of MA on LA. Such effects during adolescence do not indicate the developmental effects of adolescent MA exposure on later adulthood anxiety-related behaviour. Additionally, measuring LA alone is not sufficient to adequately determine this effect on anxiety because of the stimulant effects of MA on motor activity (Asser & Taba, 2015). These factors make it difficult to truly determine the effects of MA on anxiety using only motor activity measures. Therefore, it would be advisable to utilise additional measures of anxiety that are less likely to reflect general motor activity.

Another factor to consider is the age during adolescence investigated in this literature on anxiety effects. Some authors investigated MA-induced anxiety effects in adolescence outside of the period of periadolescence. Yet research investigating WM effects presented above suggests that peri-adolescents may be more vulnerable to the effects of MA than adolescents of other ages. Therefore, it would be beneficial to investigate the long-term effects on anxiety of repeated peri-adolescent exposure to MA.

To summarise, while not conclusive, the bulk of the existing literature suggests that adolescents are particularly vulnerable to the long-term anxiety effects of MA use.

While research is currently lacking, in one article there is an account of the long-term effects of cannabinoid exposure during adolescence versus adulthood. Chronic adolescent cannabinoid exposure led to poorer WM and more long-term anxiety-related behaviour (three weeks post-treatment) relative to exposure during adulthood (O'Shea, Singh, McGregor, & Mallet, 2004). As endocannabinoid signalling is known to interact with and regulate mesocorticolimbic dopamine (DA) activity and subsequent effects on behaviour (Corcoran, Roche, & Finn, 2015), DA being a predominant target of MA-induced effects (Ballester, Valentine, & Sofuoglu, 2017; Nickell, Siripurapu, Vartak, Crooks, & Dwoskin, 2014), MA may produce a similar effect.

There is currently insufficient research to determine the differential long-term effects of adolescent versus adult MA exposure (Luikinga et al., 2018). Nevertheless, an implication of the presented findings is that a specific period may exist when the brain is vulnerable to drug-induced changes, producing enduring behavioural deficits. In addition, there has been evidence of

heightened anxiety (King, Alicata, Cloak, & Chang, 2010b) and deficits in WM (King, Alicata, Cloak, & Chang, 2010a) behaviour in 4- to 11-month abstinent human adolescent MA users compared with healthy controls. Although these were not longitudinal studies evaluating the long-term effects of adolescent MA use on anxiety and WM, they nevertheless indicated the possibility of a long-term effect in humans. Therefore, based on this human evidence, and lack of research in this field, further research is warranted to determine these effects.

Neural Mechanism of Anxiety and Spatial WM Effects of MA

Research to date has not identified a neural mechanism for long-term neurobiological changes that explain the role of age in determining the effects of MA use (Luikinga et al., 2018). To understand whether adolescents may be more vulnerable to insult from external substances both physiologically and behaviourally, it is necessary to explore three central points. These are: the mechanism of MA-induced neural effects; where in the brain these effects occur, and how these relate to adolescent brain development and behaviour (Luikinga et al., 2018). If MA affects brain regions that are developing during adolescence it may explain potential behavioural differences between those initiating MA use during adolescence versus adulthood. This would also start to provide insight into potential treatment targets. Accordingly, the present work focuses on anxiety and [spatial] WM effects. The following discussion details these central points.

Neurotoxic effects of MA result in neuronal changes in certain brain regions, involving particular neurotransmitter systems (Luikinga et al., 2018). These effects are the result of acute and chronic use (Taylor, Lewis, & Olive, 2013). Various neurotransmitters and hormones are implicated in mediating the acute and chronic effects of MA use, such as gamma aminobutyric acid (GABA), glutamate, acetylcholine (ACh) and some stress hormones (Ballester et al., 2017). However, these have been overshadowed in the literature by the monoamines (Ballester et al., 2017). MA's pharmacological and behavioural effects are predominantly via monoamines, particularly DA (Ballester et al., 2017; Nickell et al., 2014). Acutely there is an increase in synaptic DA in the mesocorticolimbic DA system, mainly involving the ventral tegmental area (VTA) DA neurons

projecting to the nucleus accumbens (NAc) and PFC (Ballester et al., 2017). This action is primarily known for the reinforcing effects of MA use (Panenka et al., 2013).

Research has also demonstrated that human participants with MA-induced psychosis revealed significantly reduced amygdala and hippocampal volumes relative to healthy controls (Orikabe et al., 2011). Additionally, MA has been indicated to impact DA systems in these regions. For example, there has been suggested involvement of DA receptors in the amygdala in both MA-dependent and healthy human participants, as evidenced through positron emission tomography that determined amygdala DA receptor availability (Okita et al., 2016). Research has also illustrated that activation of hippocampal dopaminergic receptors mediated MA-induced increases in synaptic transmission, as shown in mice hippocampal slices (Swant, Chirwa, Stanwood, & Khoshbouei, 2010).

In addition to primary dopaminergic involvement, the activity of the DA system is modulated by several neurotransmitters (Zarrindast & Khakpai, 2015). Of relevance to potential treatment, this includes the opioid system. For example, VTA opioid receptors have been seen to indirectly regulate DA activity in the mesolimbic DA system (Ford, Mark, & Williams, 2006; Johnson & North, 1992). Amphetamine has been seen to produce an increase in endogenous opioid levels in the NAc (Olive, Koenig, Nannini, & Hodge, 2001; Wiskerke et al., 2011). More directly, MA has been demonstrated to activate opioid receptors (Chiu, Ma, & Ho, 2006). Chronic use of MA is associated with DA hypofunction in reward circuitry (Ballester et al., 2017) and neuroadaptations in the opioid system amongst other neurotransmitter systems (Georgiou et al., 2016). For example, the repeated activation of opioid receptors through prolonged MA treatment has led to receptor desensitisation (Chiu et al., 2006). This indicates that both the DA system and the opioid system may be relevant targets in the treatment of MA.

Anxiety and memory functions are associated with the aforementioned brain regions and neurotransmitter systems targeted by MA. For example, research evidence has indicated a role of the DA system in the PFC, VTA, NAc, amygdala, and hippocampus in anxiety-related behaviour (Zarrindast & Khakpai, 2015). Tu et al. (2019) illustrated the involvement of DA in the NAc in both

anxiety and spatial WM. More specifically, MA withdrawal after repeated administration effected DA in the NAc, producing spatial learning and memory impairment, and anxiety. Deletion of D1 and D2 receptors in the NAc exacerbated these spatial learning and memory deficits and anxiety-related behaviours, respectively (Tu et al., 2019).

Research also suggests the involvement of DA in the PFC and spatial WM. For example, chronic stress-induced DA insufficiency in the PFC has produced spatial WM impairments (Mizoguchi et al., 2000). Although this was not a result of drug use, it is comparable to chronic MA-associated DA-hypofunction (Ballester et al., 2017). Additional research has demonstrated that excessive DA transmission using a D1 agonist in the PFC has produced acute, dose-related spatial WM impairment, which was reversible using antagonist treatment (Zahrt, Taylor, Mathew, & Arnsten, 1997). While not related to the effects of exogenous compounds, it is akin to acute MA-associated DA-hyperfunction (Ballester et al., 2017). These findings are consistent with other research illustrating the involvement of PFC DA function in spatial WM (Williams & Castner, 2006).

While not with respect to DA, research has also demonstrated the involvement of the hippocampus in spatial WM. For example, repeated MA treatment has produced spatial WM impairment through decreases in NMDA receptor binding in regions of the hippocampus (Lee et al., 2011), and through a dysfunctional extracellular signal-regulated kinase1/2 pathway in the hippocampus (Nagai et al., 2007).

Other research has demonstrated the involvement of the opioid system in anxiety and WM behaviours. For example, regarding anxiety-related behaviours, opioidergic involvement in the ventral hippocampus and the NAc has been demonstrated (Zarrindast, Babapoor-Farrokhran, Babapoor-Farrokhran, & Rezayof, 2008). Additionally, mu-opioid receptors in the dorsal hippocampal areas (Solati, Zarrindast, & Salari, 2010) and delta opioid receptors in the central amygdala (Randall-Thompson, Pescatore, & Unterwald, 2010) have been demonstrated in anxiety-related behaviours. Hippocampal CA3 m-opioid receptors have been associated with spatial memory acquisition and retrieval (Meilandt, Barea-Rodriguez, Harvey, & Martinez, 2004). Wall

and Messier (2002) replicated findings of one of their prior studies (Wall & Messier, 2000), illustrating consistent findings of the involvement of the k-opioid receptors in the PFC in both WM and anxiety-related behaviour.

The evidence consistently highlights particular neuro-anatomical regions and neurotransmitter systems as key to both anxiety and WM function- two major effects of MA.

The final central point focuses on adolescent vulnerability to the developmental effects of MA. Those brain regions and neurotransmitter systems affected by MA use, which are also involved in anxiety and memory, are especially relevant to adolescent brain development and behaviour. The reorganisation of the brain during adolescence leaves it vulnerable to the negative impact of environmental influences (Konrad, Firk, & Uhlhaas, 2013), such as drugs, on development and behaviour (Andersen, 2003; Spear, 2000). Furthermore, MA is known to have major effects on specific regions of the developing adolescent brain (Luikinga et al., 2018).

This reorganisation of the adolescent brain is now discussed. As shown by investigations of adolescent brain development, there are extensive emotional and cognitive alterations that occur during adolescence (Konrad et al., 2013). In particular, there are significant developmental changes in the PFC, NAc, hippocampus (Arain et al., 2013; Luikinga et al., 2018), and the amygdala (Arain et al., 2013). Animal studies have shown excess neuronal development and connections in early adolescent limbic and pre-frontal brain regions, with synaptic pruning during later adolescence particularly in the NAc, amygdala and PFC (Casey, Jones, & Hare, 2008). Significant changes also occur in the dopaminergic system (Wahlstrom et al., 2010), such as alterations in DA neuronal density and receptor distribution (Luikinga et al., 2018). Factors which disturb or alter neural activity in these developing brain regions are associated with cognitive deficits and increased anxiety-related behaviours (Luikinga et al., 2018).

Certain brain areas and neurotransmitter systems involved in anxiety and memory function and MA effects are still developing during adolescence. Therefore, it is important to find treatment options that can mitigate potential developmental effects on anxiety and memory behaviours of adolescent exposure to MA.

MA Treatment: Naltrexone's Potential to Mitigate Long-Term Developmental Effects

Current treatment options for MA dependence have limited efficacy in clinical practice (Courtney & Ray, 2014; Petit et al., 2012). No approved pharmacotherapeutic agents yet exist due to inconsistent efficacy in clinical trials (Ballester et al., 2017). This indicates an obvious need to investigate alternative treatment options, particularly novel targets aimed at the prevention of potential adolescent developmental effects of MA. Naltrexone, a non-selective opioid antagonist (Dimatelis, Russell, Stein, & Daniels, 2012), has been suggested as a pharmacotherapeutic agent in the treatment of MA dependence (Dimatelis et al., 2012; Morley, Cornish, Faingold, Wood, & Haber, 2017). This is because MA has illustrated agonistic action at opioid receptors (Chiu et al., 2006) which has been shown to exacerbate amphetamine-induced increases in synaptic DA levels (Schad, Justice, & Holtzman, 2002).

Naltrexone has been shown to interact with the pharmacodynamic effects of amphetamines. For example, naltrexone pre-treatment in rats significantly reduced acute amphetamine-induced DA release 10 days after chronic amphetamine administration (10 daily 2mg/kg injections) relative to placebo. This demonstrated an indirect influence of naltrexone on amphetamine-induced DA agonism (Jayaram-Lindstrom et al., 2017). Naltrexone's demonstrated behavioural effects include: reduced MA-induced behavioural sensitisation (Chiu, Ma, & Ho, 2005); reduced MA use in human participants with MA use disorder (Kohno et al., 2018), and attenuated amphetamine-induced LA (Haggkvist et al., 2011). More recently, acute administration of naltrexone in rats has demonstrated a significant reduction in cue-induced drug seeking behaviour. This suggests the therapeutic potential of naltrexone in reducing MA relapse (Guo et al., 2019).

To summarise, the published literature suggests that MA acts on brain regions and neurotransmitter systems implicated in anxiety and WM functions. These have been shown to overlap heavily with brain regions and systems that are still developing in adolescence. Naltrexone may attenuate the physiological action of MA and has demonstrated mitigation of MA-induced behavioural effects. Therefore, naltrexone may influence the anxiety and memory-based

behavioural effects associated with MA use. More specifically, naltrexone may attenuate potential developmental effects of adolescent MA-exposure that would otherwise impact later adulthood anxiety and memory performance.

The present research aimed to determine the effects of naltrexone on the development of heightened anxiety and memory impairments in MA-exposed adolescent rats. Potential sex differences were also assessed. If successful, whereby naltrexone reduces the deleterious effects of MA, the results may have major implications for eventual adolescent treatment of MA substance use.

Aims and Methods

Aims

There is limited research on the long-term effects of adolescent MA exposure on subsequent adulthood anxiety and memory as well as efficacy of pharmacological intervention of effects of MA. Therefore, the primary aim of the present research was to compare the effects of saline, naltrexone and MA-treated groups of rats. Specifically, it was expected that rats treated with MA would demonstrate higher spatial WM impairment and anxiety relative to those that not treated with MA. It was also expected that those being administered naltrexone will later show significantly less MA-induced spatial WM impairment and anxiety following both doses of the drug (1.0mg/kg, and 2.0 mg/kg) compared with control rats.

In order to investigate possible changes over time of the treatments, anxiety and memory were measured at two different ages; late adolescence/early adulthood (EA) or 'periadolescence', and later adulthood. Human and animal research has indicated differential sex effects of MA use, with differences observed in its neurochemical (Johansen & McFadden, 2017) neurotoxic (Bourque, Liu, Dluzen, & Di Paolo, 2011; Dluzen & Liu, 2008) and behavioural (Schindler, Bross, & Thorndike, 2002) effects between males and females. Therefore, this study also aimed to determine if exposure to MA and naltrexone affected male and female rats differently with respect to anxiety and memory, at different ages.

Methods

Subjects

The subjects comprised a total of 144 (72 males and 72 females) PVG/C Hooded rats, bred in the Animal facility of the Department of Psychology at the University of Canterbury, New Zealand. There were 12 conditions, with each condition containing 8 rats (4 males and 4 females). Multiple group comparisons were made, including females versus males, naltrexone versus controls, and comparison of two MA doses versus controls. To minimise type II error a sample size

was calculated to minimise a risk of this error type. Due to the number of conditions and treatment alternatives (8 rats per treatment group), the outcome number was 96. Similar studies have shown subject numbers between 40 to around 120 (Balcells-Olivero & Vezina, 1997; Dimatelis et al., 2012; Dixon & Hughes, 2019; Strauss, Brink, Moller, Stein, & Harvey, 2014). Due to the number of treatment groups, the present experiment required the total number of rats specified. The rats were weaned at PND30 and subsequently housed in 470x 280x 230mm plastic cages in same-sexed groups of four rats for the duration of the experiment. In each cage there was unlimited free access to food and water and all rats were maintained in an ambient temperature of 22 ± 2 degrees Celsius on a 12-hour light/dark cycle (lights on at 0800 hours). The cage conditions were adhered to for the duration of the study until the end of behavioural testing at PND100+. The rats were maintained and treated in accordance with the requirements of Parts 5 (codes of welfare) and 6 (use of animals in research, teaching and testing) of the New Zealand Animal Welfare Act, 1999. All experimental procedures were approved by the Animal Ethics Committee of the University of Canterbury before research commenced (Ref: 2019/09R). Body weights and general health of the rats were monitored from the beginning (start of drug treatment) through to the end of the research (end of behavioural testing).

Procedure 1: Drug Administration and Rationale for Doses

When the rats reached PND41, they were randomly assigned to the experimental conditions outlined in Table 1. They all received two daily intraperitoneal injections, separated by an interval of 15 minutes, for 10 consecutive days until and including PND50. For 16 rats, their first injection as isotonic saline, and their second was also saline. These rats comprised the control group. For another 32 rats their first injection was saline followed by either 1 mg/kg MA (16 rats) or 2 mg/kg MA (16 rats). Then for the remaining 48 rats, their first injection was 10 mg/kg naltrexone followed by either saline, 1 mg/kg MA or 2 mg/kg MA. All injection volumes were 2 ml/kg. The doses of MA were less than those used in some previous studies of adolescent exposure to MA (e.g. Vorhees et al., 2005; White et al., 2009). They were chosen to avoid any marked neurotoxicity, which has

been the main focus of much previous research. The higher dose of 2mg/kg has been shown to lead to higher anxiety in early adulthood, as demonstrated by longer latencies of emergence into the light compartment of a light-dark box (Peterson & Hughes, 2017). The dose of naltrexone (10mg/kg) was the same as that used by Dimatelis et al. (2012) for potentially modifying the effects of adolescent exposure to MA in rats. It is important to remember that the rats were not treated with MA or naltrexone either alone or in combinations from birth to PND40, or from PND51 until the end of the experiment.

The drugs were administered in separate injections as there was a possible risk of undesirable consequences if both drugs (naltrexone and MA) were administered in a single injection. These can include: physicochemical reactions in the mixture causing, for example, precipitation or separation, and chemical instability between the drugs causing reduced effectiveness of one or both, or the production of toxic compounds (Bentley, Heard, Collins, & Chung, 2015). While this information is from a human source it is potentially relevant to animals as well.

Table 1

Study Design for Effects of Co-Administration of Naltrexone with MA

Injection 1	Injection 2 (15 mins after injection 1)					
	0mg (control)		1.0 mg/kg MA		2.0 mg/kg MA	
	Males	Females	Males	Females	Males	Females
0mg (i.e. saline)	n=8	n=8	n=8	n=8	n=8	n=8
10 mg/kg Naltrexone	n=8	n=8	n=8	n=8	n=8	n=8
	N= 16	N= 16	N= 16	N= 16	N= 16	N= 16

Procedure 2: Behavioural Testing

Ten days after their last injection the rats experienced their first day of behavioural testing (PND60+), and their second day of testing on PND100+. This was to determine the subsequent

effects of the treatment later in adulthood- rats are usually considered adults from PND60 (Marco et al., 2011). Due to the number of rats involved in the present research it was anticipated that both behavioural testing would will take longer than one day, thereby occupying several days beyond PND60 and PND100 for some rats. The rationale for two testing ages was to determine how long the effects of both MA and naltrexone are sustained for.

Each rat experienced a test in a zero maze, a light/dark box test, and a responsiveness-to-brightness change Y-maze test on successive days. All tests were performed in one of three experimental rooms. The first two types of apparatus are commonly used to assess anxiety-related behaviours (Belzung, 1999; Hascoet, Bourin, & Nic Dhonnchadha, 2001; Shepherd, Grewal, Fletcher, Bill, & Dourish, 1994) and the last is commonly used to assess WM (Hughes & Maginnity, 2007). The testing order in each type of apparatus was randomised for individual rats at each testing age. In all tests, subjects were placed in the testing apparatus and unrestricted exploratory or free-ranging behaviours were recorded by means of a momentary time-sampling procedure, whereby responses were recorded every five seconds using a stopwatch. A small CCTV camera was attached above each testing apparatus and connected to a television screen. The screen was situated on a desk away from the apparatus to watch the testing via, to enable observation of the subjects without disturbing them.

Following completion of each rat's test the apparatus was cleaned using a 5% Express Sani solution (manufactured by Kemsol), particularly when switching between male and female testing. This was to remove confounding odour cues from each rat.

Light-Dark Box.

Apparatus. The apparatus sat on top of a 70cm-high table and consisted of a wooden box with two 30 x 25 x 25 cm (length x width x height) compartments, separated by a wooden wall. The wall had 10 x 10 cm opening in it to allow movement back and forth between the compartments, which could be opened or closed by a vertical wooden insert. The light compartment was painted white and had a hinged, clear Perspex lid to allow the compartment to be illuminated by overhead

fluorescent lighting, which could be dimmed using a dial to control the intensity of the light. The dark compartment was painted black and had a hinged wooden lid which kept the compartment dark by blocking the overhead lighting.

Procedure. The subject was placed into the dark compartment of the box with both the lid and the vertical wooden slider inserted. The wooden insert was lifted out approximately 60 seconds later, allowing the rat free access to both compartments. Subsequently, the latency (in seconds), that is how long it took the rat to move into the light compartment (all four feet), was recorded. At the onset of every five seconds thereafter it was observed which compartment the rat was occupying, from which it was determined the total observations of the rat in the light compartment. It was also recorded how often it entered the light and dark compartments, from which it was calculated the percentage of light compartment entries. The subject was then removed at the end of the five-minute trial. The behaviours recorded in this test are supposed to reflect anxiety. Anxiety in the light-dark box involves conflict of approach and avoidance. Namely, choices of approaching the curiosity-arousing light compartment of the box (reflecting lower anxiety) or avoiding the aversive light compartment and occupying the darkened compartment (reflecting heightened anxiety) (Dixon & Hughes, 2019).

Zero Maze.

Apparatus. This behavioural test is used to measure anxiety, and is a modified version of the elevated plus maze (Shepherd et al., 1994). The elevated plus maze has four components originally arranged into a ‘plus’ shape. The present zero maze consisted of an elevated ring-shaped apparatus, with two enclosed sections (or ‘areas’) and two open sections. All four areas were freely accessible.

The 10cm-wide and 105cm-diameter apparatus was elevated on 70cm-tall wooden legs and was illuminated by fluorescent lighting, which was dimmed using a dial to control the intensity of the light. Each open area was connected to the ends of both enclosed areas, creating a circular runway. The two closed areas were enclosed by 25cm-high walls on both sides, and both the walls

and the floors were painted black. Both the inside and the outside of the two open areas had a 1cm-high clear Perspex lid, and the floors were painted white.

Procedure. At the beginning of the test, the rat was placed into one of the enclosed sections facing an open section. As per the light-dark box test, the latency to first emerge into the open area (all four feet) was recorded (in seconds). Every five seconds thereafter it was observed whether it was occupying an enclosed or an open area and from this observation the total number of observations in the open areas were calculated. It was also noted how many times it entered the open areas and the total number of times it entered both areas, from which it was possible to calculate the percentage of open entries. The subject was then removed at the end of the five-minute trial. Like the light-dark box, this test is also based on an approach and avoidance conflict, where exploration into open regions of the apparatus reflects lower anxiety. Conversely, remaining in the enclosed area reflects heightened anxiety (Tucker & McCabe, 2017).

Responsiveness-to-Brightness Change Test (Modified Y-Maze).

Apparatus. This test is so named as it is a modification of the usual Y-maze. Normally, the Y-maze has a long stem and two arms; a light arm and a dark arm (either left or right), that extend from the stem and the subject can chose to go down either. However, in the present research the maze was modified so there was no long stem, removing any confusion about which arm the subject was in at a given time. This is illustrated by Figure 1 below, demonstrating a short stem, a left arm and a right arm.

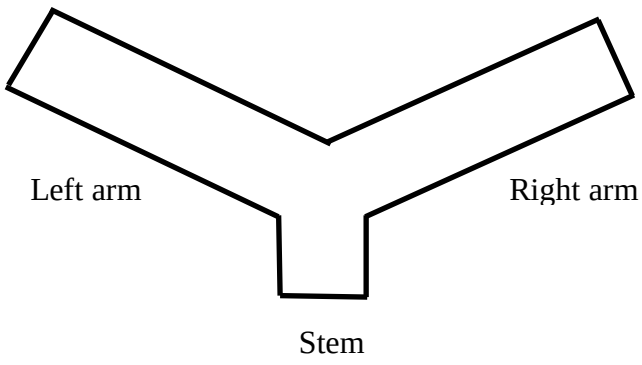

Figure 1. The modified Y-maze apparatus (original image).

The modified Y-maze was situated on top of a 70cm-high table and consisted of a clear-varnished, wooden container which was illuminated by fluorescent lighting and dimmed using a dial to control the intensity of the light. The stem was 15cm-long, while the two arms were 45cm-long and distanced with a 120° angle between them. Each the stem and the arms were each 10cm-wide and 14-cm high. The arms each contained a removable aluminium insert that was painted either black or white. The inserts occupied 40cm in length, and the full height and width of the arms. The entire maze was covered by a clear Perspex hinged lid to observe the rat and prevent it from climbing out of the apparatus.

Procedure. For the purpose of the present study, this test will be referred to as the Y-maze. Each rat had a five-minute acquisition trial, where the rat was free to explore the testing apparatus. One arm of the apparatus contained a white insert whilst the other contained a black insert. This was counter-balanced where for half of the rats the white insert was on the right, and for the other half it was on the left. The rat was then removed from the apparatus to replace the black insert with a clean black insert, and to replace the white insert with a black insert. The subject was returned to the apparatus, by being placed in the stem, for a 4.5-minute retention trial. Every five seconds it was observed which arm was being occupied; the changed/novel arm, the unchanged/familiar arm or the shortened stem. From this, the percentage of changed arm observations were able to be calculated. The number of entries into each arm was also noted over the retention trial, from which the percentage of changed arm entries could be calculated. At the end of the trial the subject was removed. The preferences recorded in this test are thought to reflect spatial WM; more specifically, recognition of which alternative has changed is thought to reflect spatial WM (Hughes & Maginnity, 2007).

All three types of apparatus were kept in the same conditions within a quiet room and illuminated by dim, overhead fluorescent lighting. Because they are all tests that require exploratory behaviour, they are based on a rat's innate curiosity to explore unfamiliar environments (Sarnyai et al., 2000).

Results

Aims

The aims of the present research were to determine whether MA has long-lasting developmental effects on anxiety and spatial WM behaviour, particularly in later adulthood. It was also to determine whether naltrexone mitigates any MA-induced later heightened anxiety and deficits in spatial WM. Analyses were conducted for behavioural testing that occurred at both PND60+ and PND100+ following treatment at PND41-50, and to calculate sex differences.

Analyses

Analyses were conducted using the StatView statistical software programme for Macintosh computers. For each testing age, all data were subjected to separate 6 (drug treatment) x 2 (sex) ANOVAs. For main effects, the significance of differences between specific groups were assessed with Scheffe post hoc tests ($p<0.05$). Significant ANOVA results for the drug treatment are included in Table 2 and significant sex effects are included in Table 3. There were no significant interactions for any of the ANOVAs, so only main effects are reported.

Table 2

Means, SEMs and F Ratios for Dose Effects for Each Measure Recorded at Two Periods (PND60+ and PND100+) After Treatment, During Adolescence, with MA and Naltrexone (Separately and in Combination)

Response	Treatment group								
	1	2	3	4	5	6			
	S+S	S+N	S+1mgMA	S+2mgMA	N+1mgMA	N+2mgMA	F	df	p
Round one									
Light/dark box:									
Emerg. latency	23.50 (4.02)	23.63 (4.47)^{ab}	51.94 (11.11)^{c}	53.25 (17.97)^{*ad}	18.56 (3.90)^{cde}	60.13 (12.95)^{*be}	3.20	5,84	0.011
Obs. in light	26.06 (1.45)	25.88 (0.85)^{ab}	23.38 (1.61)	18.94 (2.53)^{*a}	22.44 (1.50)	20.38 (1.75)^{*b}	2.86	5,84	0.020
Total entries	18.63 (0.58)	18.19 (1.01)^{ab}	16.50 (1.15)^{c}	13.00 (1.21)^{*acd}	18.00 (0.80)^{de}	14.31 (0.84)^{*be}	5.14	5,84	0.0004

% light entries	51.43 (0.38)	51.36 (0.41)	50.69 (0.34)	50.45 (0.31)	50.35 (0.24)	50.72 (0.41)	2.73	5,84	0.025
Zero maze:									
Emerg. latency	116.94 (28.67)	89.88 (27.79)	71.06 (24.51)	34.56 (7.11)*	87.06 (30.18)	31.63 (15.34)*	2.39	5,84	0.044
Y maze:									
Total entries	9.44 (0.74)	8.81 (1.06)^{a}	3.94 (1.07)^{*abcd}	7.31 (1.16)^{b}	9.19 (0.90)^{c}	7.50 (1.09)^{d}	4.96	5,84	0.0005
Total obs.	33.19 (1.69)	28.19 (2.63)^{a}	14.81 (3.40)^{*abcd}	27.50 (3.62)^{b}	29.88 (2.07)^{c}	25.25 (2.75)^{*d}	6.09	5,84	<0.0001
<u>Round two</u>									
Light/dark box:									
Emerg. Latency	39.69 (17.87)	41.13 (16.19)^{d}	61.88 (15.44)	109.44 (30.13)^{*abd}	24.38 (4.77)^{a}	48.38 (12.39)^{b}	2.90	5,84	0.018
Obs. in light	24.31 (2.71)	28.31 (2.46)^{a}	20.63 (1.90)^{abc}	21.88 (3.22)^{de}	30.94 (1.74)^{be}	31.31 (2.41)^{*cd}	3.69	5,84	0.005
Zero maze:									

Emerg. latency	168.50 (26.40)	131.75 (27.79)	100.75 (20.96)[a]	165.06 (27.99)	104.50 (23.57)[b]	198.13 (27.30)[ab]	2.64	5,84	0.029

Y maze:

% changed entries	60.35 (2.32)	48.55 (5.18)[*ab]	60.18 (4.96)[ad]	59.06 (4.89)[bc]	55.24 (2.29)	48.07 (5.61)[*cd]	3.30	5,79	0.009

S+S= saline only- saline at time one and time two

N+S= naltrexone only- naltrexone at time one and saline at time two

S+1= 1mgMA only- saline at time one and 1mg/kg MA at time two

S+2= 2mgMA only- saline at time one and 2mg/kg MA at time two

N+1= naltrexone treatment- naltrexone at time one and 1mg/kg MA at time two

N+2= naltrexone treatment- naltrexone at time one and 2mg/kg MA at time two

* = significantly different from control (S + S) ($p < 0.05$)

abcde = groups with superscripts in common significantly different (from each other) ($p < 0.05$).

Table 3

Means, SEMs and F Ratios for Significant Sex Differences for Each Measure at Two Time Points (PND60+ and PND100+) After Treatment, During Adolescence, with MA and Naltrexone (Separately and in Combination)

	Males	Females	F	df	p
Round one					
Zero maze:					
Emergence latency	107.33 (16.41)	36.38 (8.81)	16.13	1,84	<0.0001
Observed in open	12.19 (1.64)	17.10 (1.24)	5.98	1,84	0.017
Total entries of both	8.81 (1.06)	14.94 (1.11)	16.31	1,84	<0.0001
Y maze:					
Total entries of both	6.10 (0.58)	9.29 (0.59)	18.16	1,84	<0.0001
Total obs. in both	23.63 (2.00)	29.31 (1.40)	7.14	1,84	0.009
Round two					
Zero maze:					
Emergence latency	179.85 (15.70)	109.71 (13.39)	12.89	1,84	0.001
Total entries of both	6.40 (1.04)	13.48 (1.27)	19.30	1,84	<0.0001
Y maze:					
Total entries of both	4.46 (0.48)	7.77 (0.41)	26.51	1,84	<0.0001

Definition of Study Groups

Groups S+S, N+S, S+1mgMA, and S+2mgMA are considered controls for the groups N+1mgMA and N+2mgMA. For the purposes of the present experiment, S+S and N+S groups will be referred to as the baseline controls, and S+1mgMA and S+2mgMA groups will be referred to as the MA controls. Due to treatment (of MA) with naltrexone, the N+1mgMA and N+2mgMA groups will be referred to as the treatment groups.

1. Light-Dark Box Results

Each rat was tested in the light/dark box twice- at two different time points. See Table 2. for significant F ratios for measures containing group differences. There were no significant sex differences across the two testing periods.

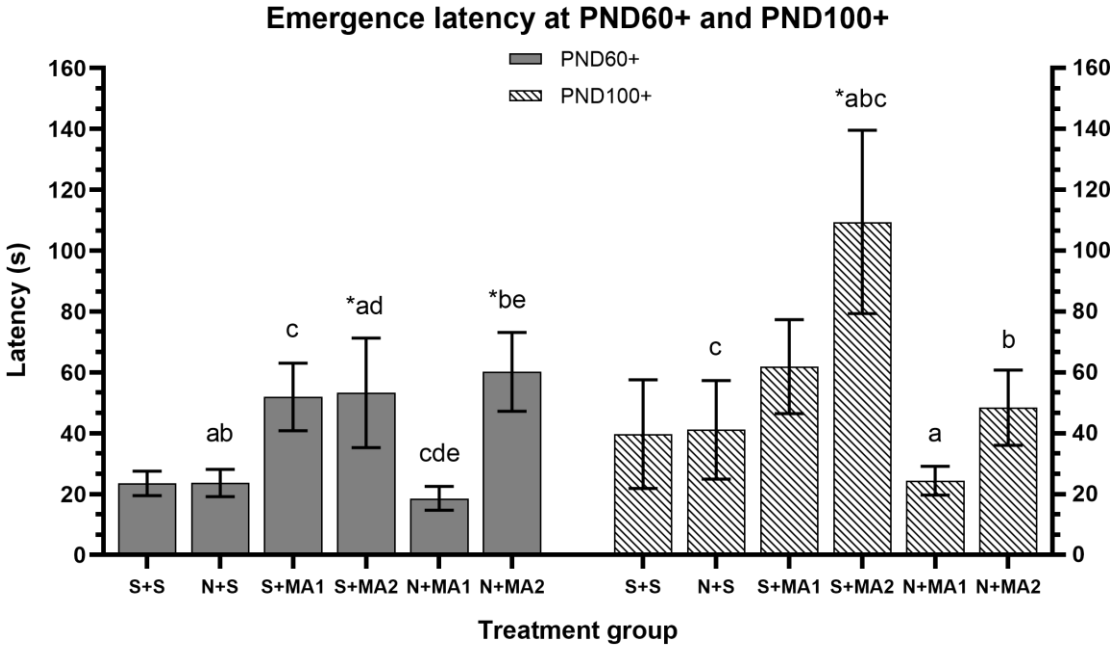

Figure 2. Mean (±S.E.M) latency to first emerge into the light compartment (in seconds) for baseline controls, MA controls, and treatment groups at PND60+ and PND100+ for male and female rats combined.

Figure 2 on the left illustrates significant differences in the average emergence latency (EL) between groups at PND60+. N+1mgMA treated rats had a significantly shorter EL than S+2mgM treated rats, which is expected due to the lower dose of MA in the treatment group. N+1mgMA rats also had a significantly shorter EL than S+1mgM rats. N+2mgMA rats were not significantly different to S+1/2mgMA treated rats. This suggests that naltrexone treatment reduced the EL of rats treated with a lower dose of MA, but not a higher dose of MA. In addition, the N+1mgM group of rats had a significantly shorter EL than the N+2mgM treated rats. Whilst this result is expected due to a lower dose of MA in the former treatment group, this result could be illustrating that naltrexone reduces EL for rats treated with a lower dose of MA relative to those treated with a higher dose of MA. N+2mgMA rats had a significantly longer EL than the S+S and N+S groups, whilst the N+1mgMA group was not significantly different to those treated with S+S. This could further illustrate that relative to a higher dose, naltrexone reduces EL for a lower dose of MA, to the equivalent to baseline EL activity. However, the results suggest that it is more likely due to MA at a 1mg dose having no effect on EL behaviour, as illustrated by those rats that were administered S+1mgMA which were not significantly different to those treated with S+S and N+S. S+2mgMA rats had a significantly longer EL than S+S and N+S treated rats. This indicates that MA treatment with a higher dose, but not a lower dose, increases EL.

Figure 2 on the right illustrates significant differences in the average EL between groups at PND100+. The N+1mgM group had a significantly shorter EL than S+2mgM, as expected due to the lower dose of MA in the treatment group. The N+2mgMA group had a significantly shorter EL than those rats that received S+2mgM. This suggests that naltrexone reduced the EL of rats treated only with a higher dose of MA but not those treated with a lower dose of MA. Those rats treated with S+2mgMA had a significantly longer EL than S+S and N+S treated rats, whilst rats treated with S+1mgMA were not significantly different to those treated with S+S or N+S. This indicates that MA treatment with a higher dose, but not a lower dose, increases EL.

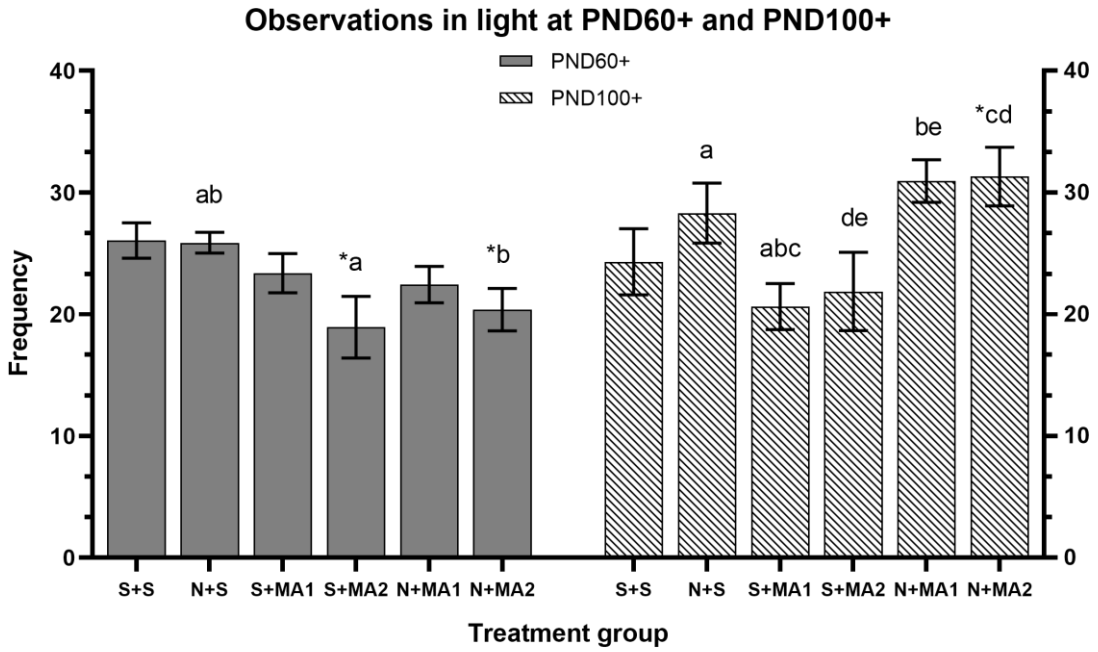

Figure 3. Mean (±S.E.M) frequency of observations in the light compartment for baseline controls, MA controls, and treatment groups at PND60+ and PND100+ for male and female rats combined.

Figure 3 on the left illustrates significant differences in the average observations in the light compartment between groups at PND60+. However, there were no significant differences between treatment groups (N+1/2mgMA) and MA control groups (S+1/2mgMA) suggesting that naltrexone did not affect the number of times rats were observed in the light compartment. Rats in the N+2mgMA treatment group were observed in the light compartment significantly less often than those rats that received S+S or N+S. This again suggests that naltrexone did not affect the number of times rats were observed in the light but is not unexpected due to the MA administered to the treatment group. That is, S+2mgMA treated rats were observed in the light significantly less often than S+S and N+S treated rats. S+1mgMA rats were not significantly different from S+S and N+S rats. This indicates that MA treatment with a higher dose, but not a lower dose, reduced observations in the light area.

Figure 3 on the right illustrates significant differences in the average light compartment observations between groups at PND100+. N+1mgM rats were observed in light significantly more often than S+2mgMA rats, which was expected due to the lower dose of MA in the treatment group.

However, N+1mgM treated rats were observed in the light significantly more often than S+1mgM treated rats. N+2mgM treated rats were also observed in the light significantly more often than S+2mgM treated rats. This suggests that naltrexone treatment increased light observations for rats treated with both doses of MA. In addition, rats administered N+2mgMA dose were observed in the light significantly more often than S+1mgMA rats, suggesting a strong effect of naltrexone in increasing the amount MA treated rats were observed in the light. Rats receiving N+2mgM were observed in the light significantly more than those receiving S+S, which may further indicate a strong effect of naltrexone in increasing light observations. It could also be reflecting the result that a 2mg dose of MA alone produced no effect on light observation behaviour, where S+2mgMA rats were not significantly different to S+S and N+S rats. S+1mgM rats were observed in the light significantly less than S+N rats, but not S+S rats. This indicates that MA treatment with a lower dose has mixed effects on 'observations in light' behaviour, and MA treatment with a higher dose has no effect on observation in light behaviour.

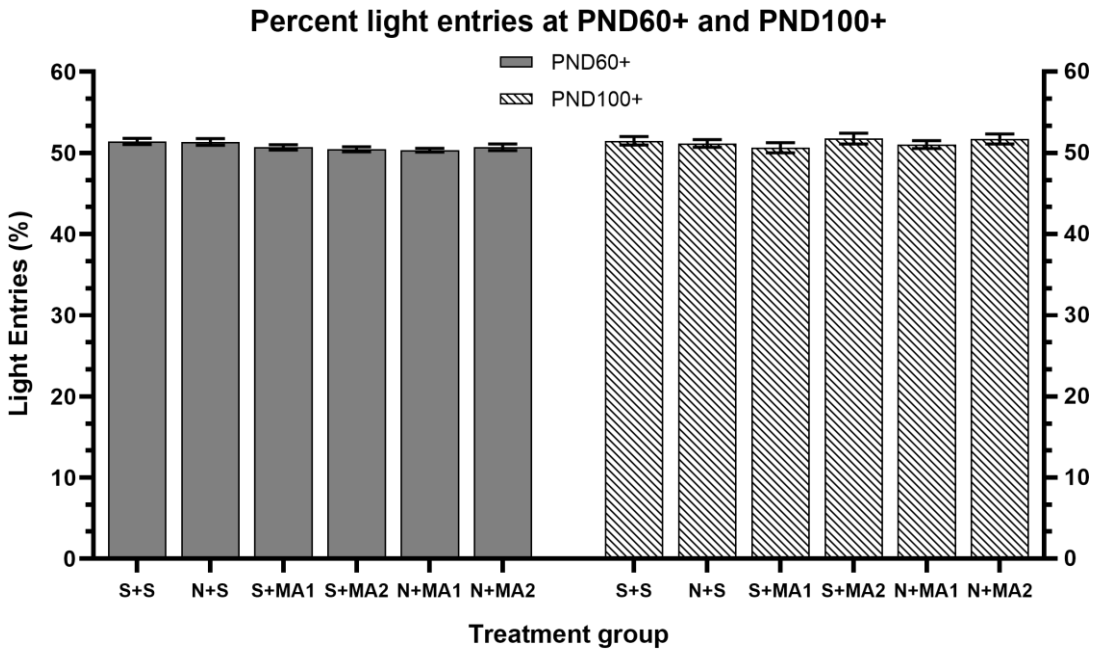

Figure 4. Mean (±S.E.M) percent entries into the light compartment for baseline controls, MA controls, and treatment groups for PND60+ and PND100+ male and female rats combined.

Although the F ratio indicated a statistically significant overall effect, it is clear from inspection of Figure 4 that differences between the means for specific groups were so small that they are of little behavioural significance. It seems likely that this situation arose from the large numbers of rats in each group that achieved a score of 50%.

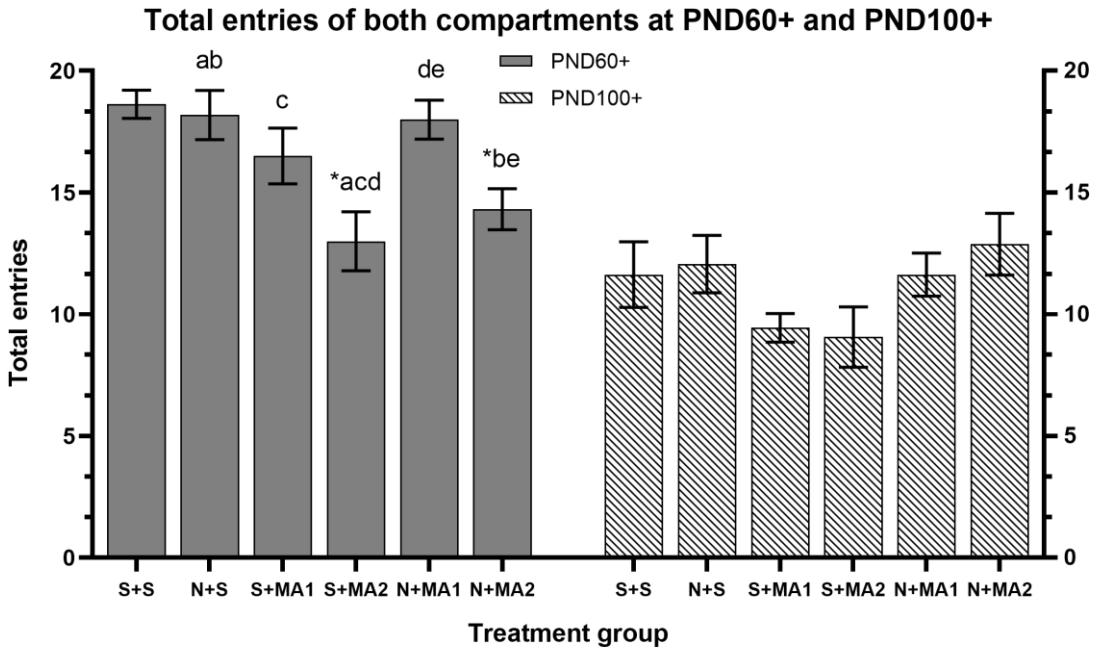

Figure 5. Mean (±S.E.M) total number of entries into both compartments of the light/dark box for baseline controls, MA controls, and treatment groups for PND60+ and PND100+ male and female rats combined.

Figure 5 on the left illustrates significant differences in the average total entries of both compartments between groups at PND60+. However, there were no significant differences between treatment groups (N+1/2mgMA) and MA control groups (S+1/2mgMA). This suggests that naltrexone did not influence the total number of entries into both compartments for rats treated with either a lower or a higher MA dose. An exception is where rats treated with N+1mgM had significantly greater total entries than rats treated with S+2mgM. However, this is an expected result due to the lower dose of MA in the treatment group. Additionally, rats treated with N+2mgM had significantly fewer total entries than those treated with only S+S and N+S. This again indicates that naltrexone did not affect the amount rats were observed in the light but is not unexpected due to the MA administered to the treatment group. That is, S+2mgM treated rats groups had significantly lower total entries than S+S and N+S. S+1mgMA treated rats were not significantly different to those treated with S+S and N+S. This indicates that MA treatment with a higher dose, but not a lower dose, reduces total entries into both areas. N+1mgMA rats had a higher total number of

entries than N+2mgMA rats which indicates naltrexone increases the total number of entries for rats treated with a lower dose of MA relative to those treated with a higher dose of MA. This is surprising as the results suggested that naltrexone had no effect on the total entries into both compartments for rats treated with either dose of MA. However, this finding is consistent with the result that S+1mgM rats had significantly higher total entries than S+2mgM treated rats.

Figure 5 on the right illustrates that there were no significant differences in the total entries into both compartments between groups in the light/dark box test at PND100+.

2. Zero Maze Results

Each rat was tested in the zero maze twice- at two different time points. See Table 2. for significant F ratios for measures containing group differences, and Table 3. for significant F ratios for measures containing sex differences across the two testing periods.

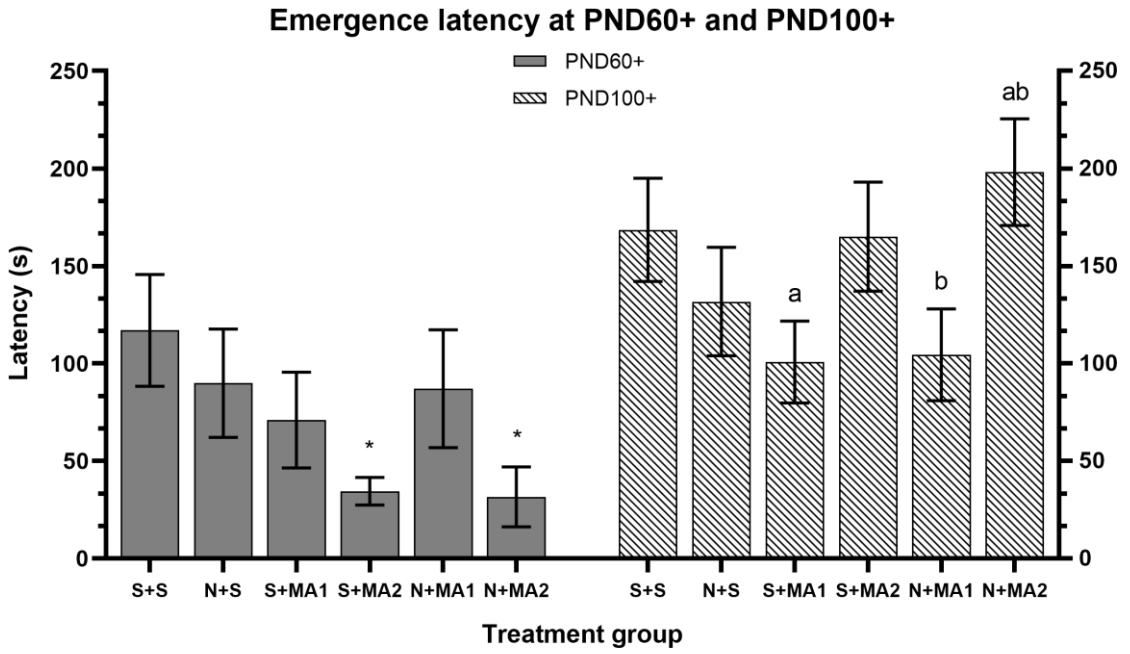

Figure 6. Mean (±S.E.M) latency to first emerge into an open area (in seconds) for baseline controls, MA controls, and treatment groups for PND60+ and PND100+ male and female rats combined.

Figure 6 on the left indicates significant differences in the average EL between groups at PND60+. However, there were no significant differences between treatment groups (N+1/2mgMA) and MA control groups (S+1/2mgMA) suggesting that naltrexone did not influence EL behaviour for rats treated with either a lower or a higher MA dose. Surprisingly, rats treated with N+2mgM had a significantly shorter EL than those treated with S+S. This is likely because MA at this dose (S+2mgMA) had a significantly shorter EL than S+S rats. However, the S+2mgMA group was not significantly different in EL behaviour to the N+S group. This suggests that MA treatment with a higher dose has mixed effects on EL behaviour. S+1mgMA rats were not significantly different to

S+S and N+S rats, suggesting that MA treatment with a lower dose had no effect on EL behaviour. Additionally, there was a significant overall effect of sex at PND60+, where female rats had a shorter EL on average than males.

Figure 6 on the right illustrates significant differences in the average EL between groups at PND100+. As at PND60+, there were no significant differences between treatment groups (N+1/2mgMA) and MA control groups (S+1/2mgMA). An exception is where rats treated with N+2mgMA had a significantly longer EL than rats treated with S+1mgMA, however this is an expected result from the higher dose of MA administered to the treatment group. Again, this suggests that naltrexone did not affect EL behaviour for rats treated with either a lower or a higher MA dose. N+1mgM rats had a significantly shorter EL than N+2mgMA rats, which indicates naltrexone decreases the total number of entries for rats treated with a lower dose of MA relative to those treated with a higher dose of MA. This is surprising as naltrexone demonstrated no effect on the total entries into both compartments for rats treated with either dose of MA, nor is it consistent with the effect of MA seen at either dose. That is, the results suggest that MA treatment at both doses had no effect on EL behaviour as S+1mgMA and S+2mgMA groups were not significantly different to S+S and N+S groups. Consistent with at PND60+, females had a significantly shorter EL than males.

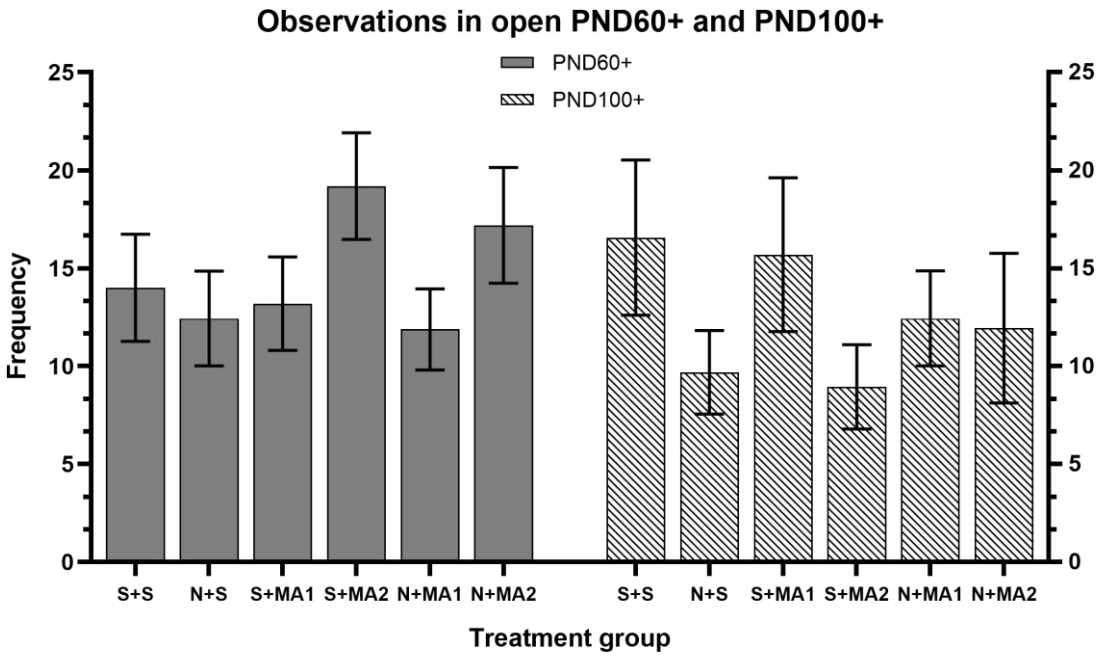

Figure 7. Mean (±S.E.M) frequency of observed open area occupation for baseline controls, MA controls, and treatment groups for PND60+ and PND100+ male and female rats combined.

Figure 7 on the left and right illustrates that there was no significant overall effect for observations in the open area in the zero-maze test at PND60+ or PND100+. However, there was a significant overall effect of sex at PND60+ where female rats were observed in the open area significantly more than male rats.

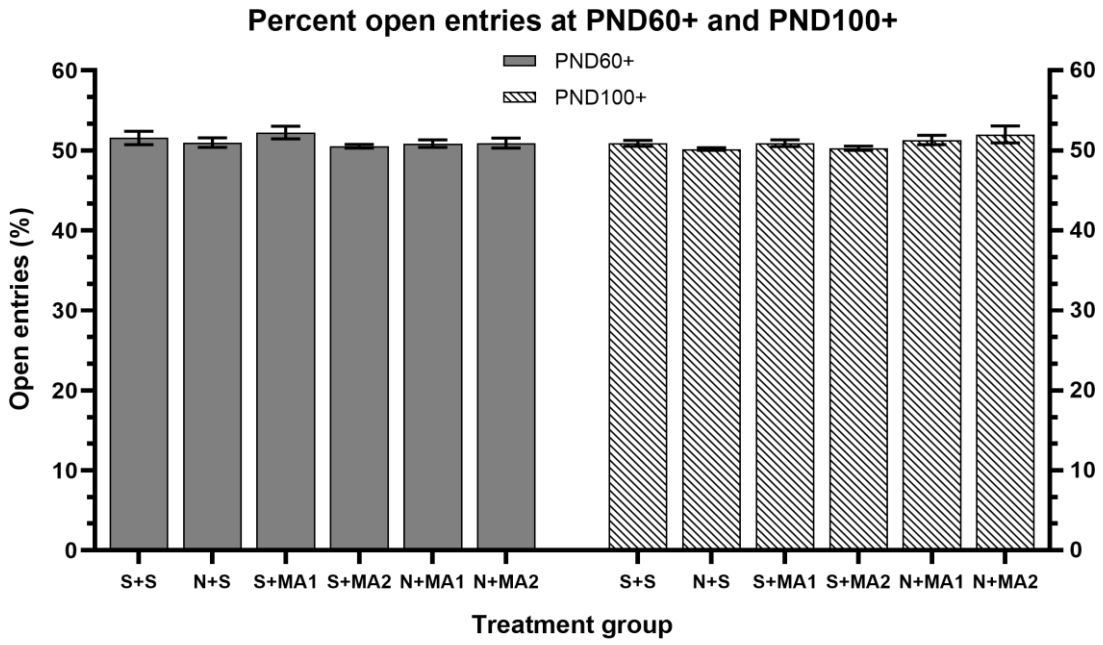

Figure 8. Mean (±S.E.M) percent entries into an open area for baseline controls, MA controls, and treatment groups for PND60+ and PND100+ male and female rats combined.

Figure 8 on the left and right indicates that there was no significant overall effect for the percentage of open area entries in the zero-maze test at PND60+ or PND100+.

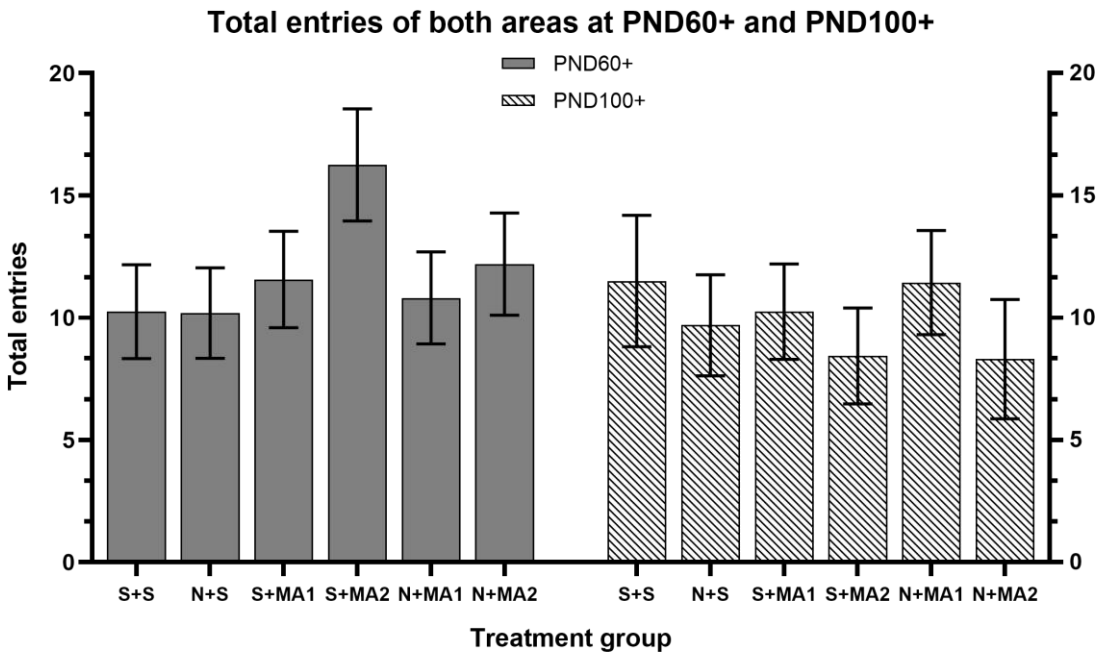

Figure 9. Mean (±S.E.M) total entries into both areas in the zero-maze test for baseline controls, MA controls, and treatment groups for PND60+ and PND100+ male and female rats combined.

Figure 9 on the left and right illustrates that there was no significant overall effect for the total entries of both compartment in the zero-maze test at PND60+ or PND100+. However, there was a significant overall effect of sex at both PND60+ and PND100+ where female rats had a significantly higher total entries of both areas than male rats.

3. Y-Maze Results

Each rat was tested in the Y-maze twice- at two different time points. See Table 2 for significant F ratios for measures containing group differences, and Table 3 for significant F ratios for measures containing sex differences across the two testing periods.

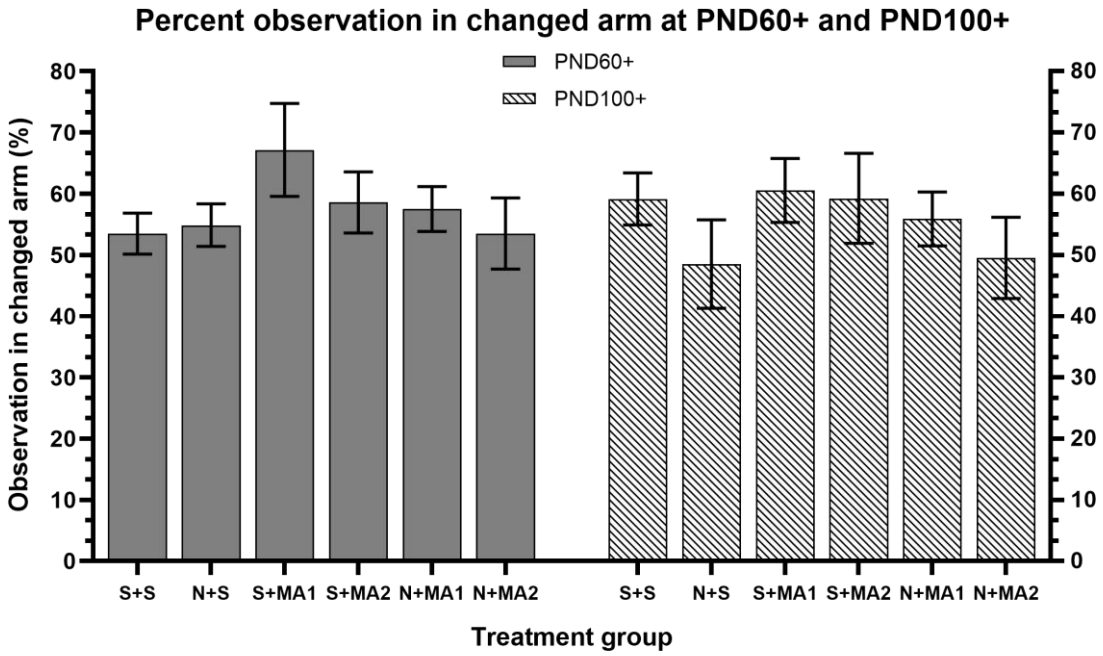

Figure 10. Mean (±S.E.M) percent observation in the changed arm in the modified Y-maze test for baseline controls, MA controls, and treatment groups for PND60+ and PND100+ male and female rats combined.

Figure 10 on the left and right illustrates that there was no significant overall effect for the percent observation in the changed arm in the adjusted Y maze at both PND60+ or PND100+.

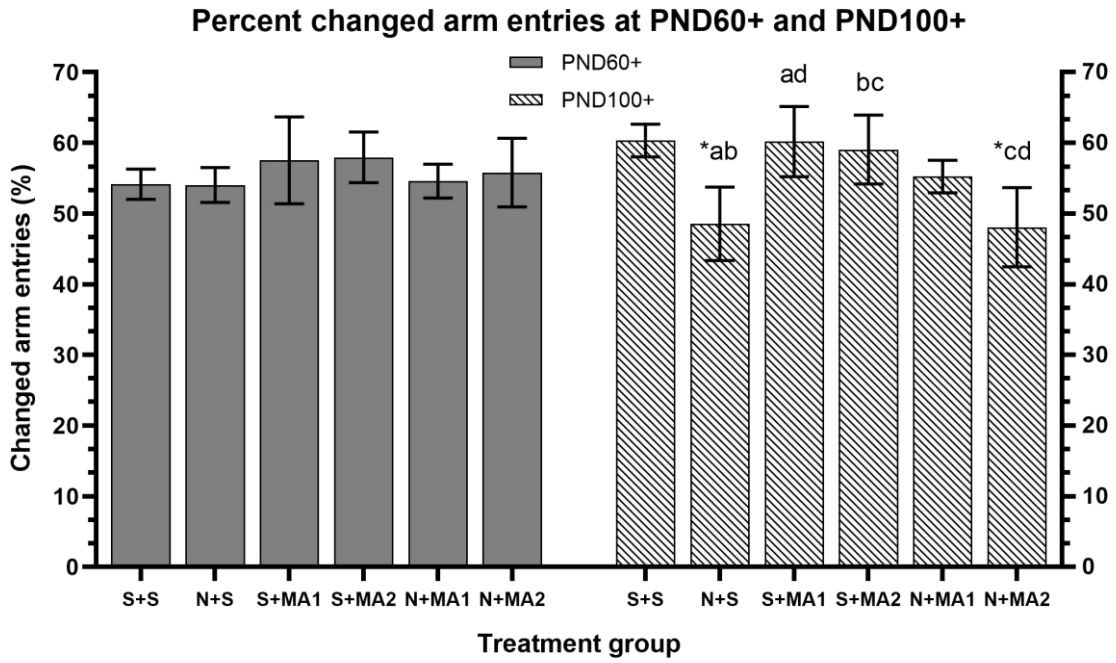

Figure 11. Mean (±S.E.M) percent changed arm entries in the adjusted Y-maze test for baseline controls, MA controls, and treatment groups for PND60+ and PND100+ male and female rats combined.

Figure 11 on the left illustrates that there was no significant overall effect for the percent changed arm entries in the adjusted Y maze at PND60+.

Figure 11 on the right demonstrates significant differences in the average percentage of changed arm entries between groups at PND100+. Rats that received N+2mgMA had significantly lower percent 'changed entries' than rats that received S+2mgMA and S+1mgMA. This suggests that naltrexone reduced the percentage of entries into the changed arm for rats treated with a higher dose of MA. Results also suggest that treatment with naltrexone did not affect the percent of changed arm entries for rats treated with a lower dose of MA, as N+1mgMA treated rats did not differ significantly to those treated with S+1mgMA. Rats administered N+2mgM and N+S had significantly lower percent changed entries than S+S. These outcomes are consistent with the finding where naltrexone treatment reduced the percentage of changed arm entries (for a higher dose of MA). S+1mgM and S+2mgM groups had significantly higher percent changed entries than

N+S rats and did not differ significantly to S+S treated rats. This suggests a mixed effect of MA both increasing the percentage of changed entries and having no effect on this behaviour.

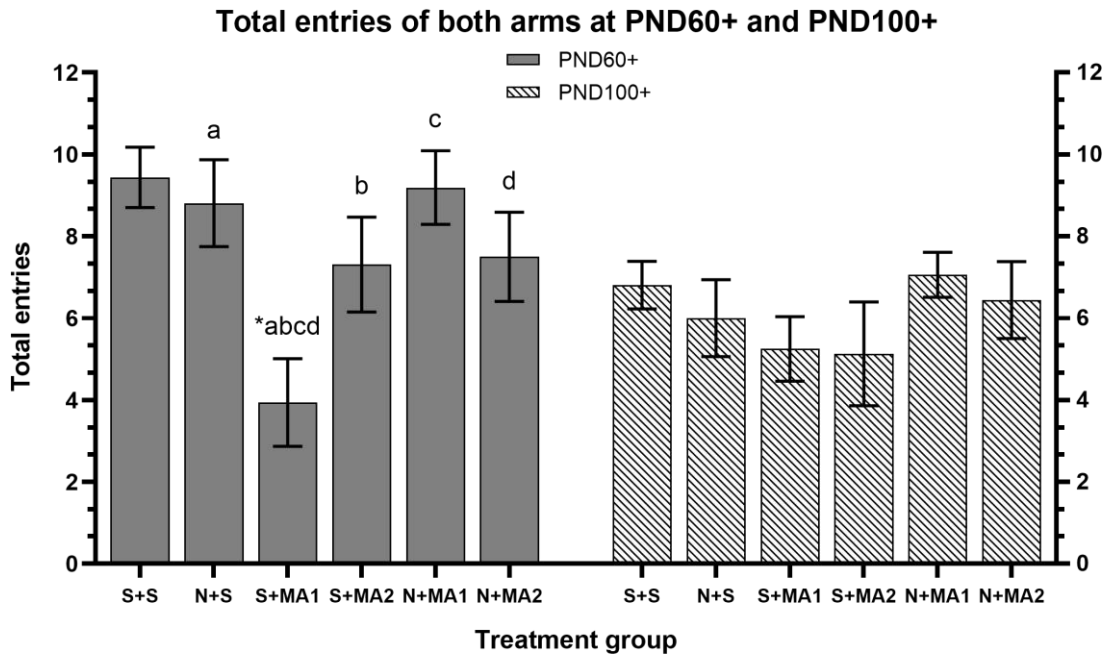

Figure 12. Mean (±S.E.M) total entries of both unchanged and changed arm entries in the adjusted Y-maze test for baseline controls, MA controls, and treatment groups for PND60+ and PND100+ male and female rats combined.

Figure 12 on the left illustrates significant differences in the total entries of both arms between groups at PND60+. N+1mgM rats made a significantly higher total number of entries into 'both' than rats administered S+1mgMA. Rats that received N+2mgM did not differ significantly from S+2mgM treated rats but they did have significantly higher total entries of both than those administered S+1mgMA. These outcomes illustrate that naltrexone treatment increased the total number of entries into both arms for rats treated with either dose of MA. S+1mgM treated rats had significantly fewer total entries than S+2mgM rats, which suggests that a lower dose of MA reduces the total number of entries into each arm relative to rats treated with a higher dose of MA. This is consistent with the effect of MA seen where results indicate that MA of a lower dose reduces the total number of entries into each arm, whilst also suggesting that MA at a higher dose has no effect on this behaviour. That is, S+1mgM rats had significantly fewer total entries of both than S+S and N+S treated rats, whilst S+2mgMA rats were not significantly different to S+S and N+S.

Additionally, there was a significant effect of sex, where females made a significantly higher total number of entries of both arms than males.

Figure 12 on the right illustrates that there was no significant overall effect for total entries of both arms in the adjusted Y maze at PND100+. However, consistent with at PND60+, there was a significant of sex where female rats made a significantly higher number of total entries of both arms than male rats.

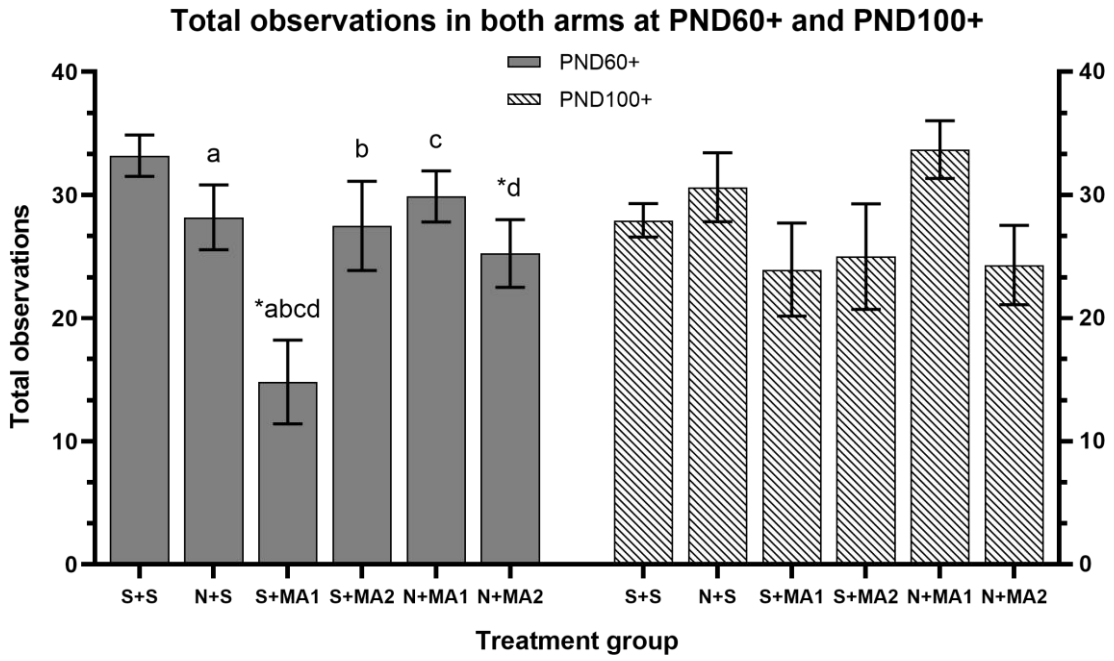

Figure 13. Mean (±S.E.M) total observations in both unchanged and changed arms in the adjusted Y-maze test for baseline controls, MA controls, and treatment groups for PND60+ and PND100+ male and female rats combined.

Figure 13 on the left demonstrates significant differences in the total observations in both arms between groups at PND60+. N+1mgM treated rats had a significantly higher total observation in 'both' than rats administered S+1mgM. Rats that received N+2mgM did not differ significantly from S+2mgMA rats, however they did have a significantly higher total observation in both than rats administered S+1mgMA. This suggests that naltrexone increased the total observations in both arms for rats treated with either dose of MA. Surprisingly, S+1mgM treated rats had significantly fewer total observations in both than those treated with S+2mgM, indicating that a lower dose of MA reduced the total observations in each arm relative to rats treated with a higher dose of MA. However, this is consistent with the effect of MA seen. S+1mgM rats had significantly fewer total obs. in both than S+S and, S+N groups, whilst S+2mgMA administered rats were not significantly different to S+S or N+S rats. This also illustrates that a lower dose of MA reduced the total observations in both arms, whilst a higher dose of MA has no effect on this behaviour. N+2mgM treated rats had significantly fewer total observations than S+S rats and were not significantly

different in this behaviour to N+S treated rats. The former result is not consistent with the suggested effect of naltrexone or a 2mg dose of MA. The latter outcome again suggests that a higher dose of MA had no effect on this behaviour, as it is consistent with the finding that S+2mgMA rats were not significantly different to N+S rats. Additionally, there was a significant effect of sex where females had a significantly higher total observation in both arms than males.

Figure 13 on the right illustrates that there was no significant overall effect for the total observations in both arms in the adjusted Y maze at PND100+.

Discussion of Results

In the present investigation, adolescent rats were administered once-daily intraperitoneal injections of saline or naltrexone (10mg/kg), saline or MA (1mg/kg or 2mg/kg) and naltrexone and MA combined for ten days from PND41-50. All rats were later tested on measures of anxiety and spatial WM at PND60+ and PND100+ to determine any long-term effects of drug administration.

Overall, it appears that adolescent exposure to naltrexone and MA did not affect either later anxiety or WM behaviour. While the general pattern of results suggests no effect on anxiety and WM, there was no clear behavioural effect of either MA or naltrexone on their own, complicating the interpretation of these results. See Table 4 in the 'General Discussion' for a summary of the effect of naltrexone and MA on anxiety and WM behaviour for each MA dose and testing age.

In contrast, a very clear pattern emerged regarding the effect of sex. For measures where there were significant differences, results suggest that females demonstrated consistently lower anxiety-related behaviour than males. This indicates that male rats may be more prone to anxiety than females.

The following will discuss the results with respect to anxiety and WM and will answer questions pertaining to all relevant types of group comparisons for each test. This discussion will be based on measures from each test with significant F ratios.

Question 1: Does Naltrexone Alone Make a Difference to Behaviour?

During the Y-maze test at PND100+ the naltrexone-only group demonstrated a significantly lower percentage of changed arm entries than the saline-only group. This indicates that naltrexone impaired WM. Otherwise, for all three tests the saline-only treated rats were not significantly different in anxiety or WM behaviour to the naltrexone-only treated rats. This indicates that overall naltrexone did not affect anxiety significantly different from saline. This result is expected, as naltrexone did not modify the effect of MA and was not expected itself to reduce anxiety.

1. Light-Dark Box

Of the five measures at each testing age, groups demonstrated differences in anxiety-related responses for total entries of both compartments at PND60+, and for EL and observations in light at both PND60+ and PND100+.

Question 2: Does MA Alone Make a Difference to Behaviour?

The results indicate that the saline and naltrexone-only groups did not differ in anxiety-related behaviour from the 1mgMA group at either age. However, at PND100+, the 1mgMA group demonstrated higher anxiety, as indicated by fewer observations in the light than the naltrexone-only group but not the saline-only group. Generally, this indicates that a lower dose of MA did not have an anxiety heightening effect at EA or, of particular interest, at later adulthood.

Outcomes suggest that the saline and naltrexone-only groups were less anxious than the 2mgMA group at both ages. However, at PND100+ there were no significant differences in the total observations in light between the 2mgMA group and the saline and naltrexone-only groups, indicating no group differences in anxiety. It is difficult to determine whether a higher dose of MA had an anxiety heightening effect in later adulthood from the results at this test, but the results indicate that a higher dose of MA does have an anxiety heightening effect in EA.

Question 3: Does Naltrexone Alter the Effect of MA?

The results indicate that at EA there was inconsistency in the effect of naltrexone when paired with a lower dose of MA; however, they generally illustrate a lack of any influence of naltrexone. N+1mgMA treated rats demonstrated lower anxiety as indicated by a shorter EL, but also no difference in anxiety as suggested from more observations in light relative to 1mgMA (and 2mgMA) groups. A higher total number of entries of both compartments indicates that N+1mgMA rats were demonstrating lower anxiety than 2mgMA treated rats. However, this is expected with a lower dose of MA so does not truly indicate a MA-mitigating effect of naltrexone. For these reasons, and the fact that MA did not produce the expected outcome of heightened anxiety at EA, it

cannot be concluded that naltrexone demonstrated an anxiety-mitigating effect of a lower dose of MA in EA. It is therefore also unsurprising that rats treated with N+1mgMA did not differ in anxiety level to baseline (id est, saline and naltrexone only groups), likely to be because of the lack of an anxiety-inducing effect of MA.

At EA there were, however, consistent results demonstrating no difference in anxiety-related responses between N+2mgMA treated rats and rats treated with either 1mgMA or 2mgMA. This likely illustrates a lack of a MA-mitigating effect of naltrexone, as MA at this higher dose demonstrated the expected anxiety heightening effects. Also, N+2mgMA treated rats at EA demonstrated higher anxiety-related responses than saline and naltrexone only treated rats, further illustrating the anxiety-heightening effect of 2mgMA and lack of MA-mitigating effect of naltrexone. Therefore, it may be concluded that naltrexone did not demonstrate an anxiety-mitigating effect on a higher dose of MA at EA.

At later adulthood there were inconsistent results regarding the effect of naltrexone treatment of a lower dose of MA. The N+1mgMA treated rats demonstrated lower anxiety as indicated by a shorter EL than 2mgMA treated rats, but not 1mgMA treated rats. This former result is expected due to the lower dose of MA experienced by the naltrexone treated group than the 2mgMA group. This lack of a naltrexone effect indicated by the EL measure is probably due to the lack of a 1mgMA effect at later adulthood on EL behaviour. In contrast, N+1mgMA treated rats demonstrated lower anxiety as indicated by more observations in the light than 1mgMA (and 2mgMA) treated rats. The suggested anxiety-reducing effect of naltrexone seen at this measure may indicate a mitigating effect of MA-induced anxiety in later adulthood. This is because 1mgMA in later adulthood demonstrated heightened anxiety, though only relative to naltrexone alone not saline alone. Because of these inconsistencies in the effect of MA and naltrexone, it cannot be concluded with confidence that naltrexone demonstrated an anxiety-mitigating effect of a lower dose of MA at later adulthood. It is also therefore unsurprising that rats treated with N+1mgMA did not differ in anxiety levels to those treated with saline and naltrexone alone- possibly because of the lack of anxiety-inducing effect of MA.

In contrast, there were consistent results demonstrating an anxiety reducing effect of naltrexone treatment of a higher dose of MA at later adulthood. Results at both significant measures indicated that N+2mgMA treated rats demonstrated lower anxiety-related responses than 2mgMA treated rats. N+2mgMA rats also demonstrated lower anxiety than 1mgMA treated rats as indicated by more observations in the light. Generally, this indicates that naltrexone had an anxiety-mitigating effect of a higher dose of MA. In addition, results for all of the significant measures indicate that the N+2mgMA group was not different in anxiety-related responses to the baseline groups treated with saline only and naltrexone-only. That is, except for the observations in light measure where the N+2mgMA rats demonstrated lower anxiety than those treated with only saline. The former result may further indicate that naltrexone reduced anxiety to the equivalent of baseline anxiety-related behaviour. The latter result could indicate that naltrexone also has an anxiolytic effect, on top of an MA-mitigating effect. However, this finding is not consistent with the outcome that naltrexone and saline treated rats did not differ in the amount they were observed in the light compartment, suggesting that naltrexone alone does not reduce anxiety.

Whilst the results above indicate an anxiety-mitigating effect of naltrexone at later adulthood, there were inconsistent results regarding the effect of a higher dose of MA. That is, EL results indicate that 2mgMA treated rats demonstrated heightened anxiety, but outcomes from the observations in light measure do not. Therefore, while based on the EL findings this is a possibility, it again cannot be concluded with confidence that naltrexone demonstrated an anxiety-mitigating effect at later adulthood of the higher dose of MA.

Question 4: Does MA Dose Make a Difference to Behaviour and Alter the Effect of Naltrexone?

a) Does MA Dose Make a Difference to Behaviour? There was no difference in anxiety-related responses between the 1mgMA and 2mgMA groups at either age. This is not consistent with the outcome that each dose of MA alone had different effects on anxiety-related responses (relative to the saline and naltrexone groups). That is, except at PND60+ when the 1mgMA treated rats demonstrated greater total entries of both compartments, indicating lower anxiety than rats treated

with 2mgMA. This is consistent with the finding that 2mgMA but not 1mgMA increased anxiety relative to saline and naltrexone, again indicating that treatment with 1mgMA produces relatively less anxiety than a higher dose of MA.

b) Does MA Dose Alter the Effect of Naltrexone? The results indicate that MA dose did alter the effect of naltrexone, only for certain measures at PND60+, where a shorter EL and higher total entries of both arms for the N+1mgMA group suggested lower anxiety-related behaviour relative to the N+2mgMA group. This is consistent with the expectation that a lower dose of MA would produce relatively lower anxiety-related responses. It is also consistent with the present findings that a lower MA dose lacked an anxiety-inducing effect, and in contrast, with the finding that a higher MA dose had an anxiety-inducing effect. However, based on the effect of naltrexone seen from the presented findings, it is difficult to truly determine whether MA dose alters the effect of naltrexone. At PND100+ there were no significant differences between N+1mgMA and N+2mgMA groups.

Overall, some results were ambiguous, making it difficult to produce a confident conclusion. However, collectively, they generally indicated two main points. Firstly, that a lower dose of MA experienced during adolescence did not have lasting effects on the rat's behavioural development at EA, but a higher dose did. In later adulthood the results point more towards a lack of a MA effect, irrespective of MA dose. Secondly, naltrexone did not demonstrate a clear mitigating effect of either EA or later adulthood anxiety. However, there remains the potential that naltrexone had a MA-mitigating effect in later adulthood.

2. Zero Maze

There were no significant differences between groups at any measure except for the EL measure at both testing ages, indicating that the treatments had different effects on anxiety-related behaviour only for this measure.

Question 2: Does MA Alone Make a Difference to Behaviour?

There were no significant differences in the EL, thus suggesting no differences in anxiety levels between the saline only and naltrexone only groups and the 1mgMA and 2mgMA groups at either EA or later adulthood. However, at PND60+, 2mgMA treated rats had a significantly shorter EL than saline-only treated rats. Contrary to expectation, this indicates that MA reduced anxiety. Regardless, these findings indicate that for no measure of anxiety in the zero maze did rats treated with MA demonstrate heightened anxiety. It also indicates that MA experienced during adolescence does not have long-lasting effects on the rats' behavioural development to produce heightened anxiety at either EA or, of interest, at later adulthood.

Question 3: Does Naltrexone Alter the Effect of MA?

Naltrexone did not demonstrate a mitigating effect of either dose of MA, for any measure, at either testing age. At PND100+ the N+2mgMA group had a longer EL, demonstrating higher anxiety than the 1mgMA group. However, this is not an unexpected outcome as the N+2mgMA treatment group received a higher dose of MA. This indicates a lack of an anxiety-reducing effect of naltrexone in the zero-maze test, particularly at later adulthood. There were also no significant differences in the emergence latencies of saline-only and naltrexone-only treated rats and those treated with N+1mgMA or N+2mgMA, indicating no differences in their anxiety levels. This is likely a result of the lack of MA-induced anxiety effect. However, at PND60+, N+2mgMA rats demonstrated lower anxiety than saline-treated rats, due to their shorter EL. This unexpected outcome is likely because of the effect of treatment with 2mgMA rather than treatment with naltrexone, as illustrated by 2mgMA rats who demonstrated lower anxiety than saline-only treated rats. Again, this indicates that a higher dose of MA reduces anxiety.

Question 4: Does MA Dose Make a Difference to Behaviour and Alter the Effect of Naltrexone?

a) Does MA Dose Make a Difference to Behaviour? Unsurprisingly, there was no significant difference in the EL between the 1mgMA group and the 2mgMA group, suggesting no difference in their anxiety-related behaviour at either testing age. This indicates that MA dose does

not make a difference to anxiety-related behaviour measured in this apparatus, suggesting that MA alone, irrespective of dose, does not produce heightened anxiety.

b) Does MA Dose Alter the Effect of Naltrexone? The only significant difference between the two naltrexone treatment groups was at PND100+, where N+2mgMA treated rats had a longer EL than N+1mgMA treated rats, suggesting that N+2mgMA rats demonstrated higher anxiety-related behaviour. This is consistent with the expectation that a higher dose of MA would produce higher anxiety levels. However, at no other measure was there a significant difference between the N+2mgMA and N+1mgMA groups. Therefore, this suggests that MA dose does not alter the effect of naltrexone.

To summarise, from the presented results it may be inferred that MA experienced during adolescence did not have long-lasting effects on the rats' behavioural development. This is with respect to anxiety as measured in the zero maze at EA or later adulthood. Therefore, it is unsurprising that naltrexone did not demonstrate an anxiety-reducing effect at either age, as there was no apparent anxiety-enhancing effect of MA for naltrexone to mitigate.

3. Y-Maze

Of the four measures at the Y-maze, there were different effects between groups for the measures 'total entries both' and 'total observations both' at PND60+, and 'percent changed entries' at PND100+.

Question 2: Does MA Alone Make a Difference to Behaviour?

A lower dose of MA produced a lasting effect on behaviour at PND60+, however this effect was mixed at PND100+. For both measures at PND60+, the results suggest that the 1mgMA group demonstrated higher anxiety levels than the saline-only and naltrexone-only groups. This indicates that a lower dose of MA produces heightened anxiety in EA. At PND100+, 1mgMA treated rats were not significantly different in the percentage of changed entries to the saline-only group, suggesting no difference in spatial WM. However, unexpectedly, the 1mgMA group produced a

significantly higher percentage of changed entries, indicating better spatial WM performance compared to the naltrexone only group. This indicates that a lower dose of MA experienced during adolescence may have lasting effects on anxiety in EA, but no long-lasting effects on WM in later adulthood. The lower dose of MA potentially enhances spatial WM ability.

A higher dose of MA was not seen to produce long-lasting anxiety heightening or memory-impairing effects on behaviour. At both ages, saline-only and naltrexone-only groups were not significantly different to 2mgMA treated rats. That is, except at PND100+, when the 2mgMA rats made a significantly higher percentage of changed entries, indicating better WM performance than the naltrexone-only group. This indicates that a higher dose of MA experienced during adolescence does not increase anxiety-related behaviour long-term at either EA, nor decrease spatial WM performance longer-term at later adulthood. A higher dose of MA may enhance spatial WM ability.

Question 3: Does Naltrexone Alter the Effect of MA?

The results at PND60+ suggest that naltrexone mitigates anxiety for a lower dose of MA, as N+1mgMA-treated rats demonstrated lower anxiety-related responses than 1mgMA-treated rats at both significant measures. These results likely reflect that naltrexone is mitigating the anxiety-increasing effect of a lower dose of MA, as 1mgMA alone at EA heightened anxiety. Naltrexone may also mitigate the anxiety-enhancing effect of 1mgMA to the equivalent of baseline anxiety-related behaviour. This is demonstrated by the result that the saline-only and naltrexone-only groups were not significantly different to N+1mgMA treated rats at either measure.

Again, at PND60+, results from both significant measures suggest that the N+2mgMA group demonstrated lower anxiety than the 1mgMA group, however demonstrated no difference in anxiety to the 2mgMA group- which was unexpected. The former result would suggest that naltrexone mitigates the effect of a higher dose of MA and produces anxiety levels lower than those resulting from a lower dose of MA alone. However, this interpretation cannot be accepted with confidence for two main reasons. The first being that the N+2mgMA rats did not demonstrate lower anxiety-related responses than the 2mgMA rats; the second being that 2mgMA alone did not

demonstrate heightened anxiety relative to the baseline control groups (saline and naltrexone-only groups). As 2mgMA did not heighten anxiety, naltrexone could not have been mitigating MA. There was also no difference between the saline or naltrexone only groups and the N+2mgMA group at these PND60+ measures. That is except that the N+2mgMA treated rats had a greater total number of observations in both arms, suggesting higher anxiety levels than saline-only, but not the naltrexone-only treated rats. Generally, these findings likely illustrate the lack of an anxiety-heightening effect from a higher dose of MA.

Together, these results indicate that naltrexone may reduce the long-term anxiety-inducing effect a lower dose, but not a higher dose of MA in EA.

Naltrexone did not reduce anxiety or memory deficits at PND100+ for rats treated with either dose of MA. The N+1mgMA treated rats were not significantly different in the percentage of changed arm entries to the 1mgMA or 2mgMA treated rats, suggesting that they did not differ in WM performance. The N+2mgMA treated rats made a significantly lower percentage of changed arm entries, indicating that they demonstrated worse WM performance than the 2mgMA and 1mgMA treated rats. In addition, there was no difference between the saline and naltrexone groups and either the N+1mgMA or N+2mgMA groups. That is except where the N+2mgMA group demonstrated greater WM deficits than the saline group (but not the naltrexone group), as suggested by their significantly lower percentage of changed arm entries. Generally, these results are consistent with the lack of effect that both doses of MA had on spatial WM behaviour, and the relative decrease in WM performance seen by treatment with only naltrexone (relative to saline alone).

Question 4: Does MA Dose Make a Difference to Behaviour and Alter the Effect of Naltrexone?

a) Does MA Dose Make a Difference to Behaviour? At both total entries and total observations at PND60+, the results indicate lower anxiety for the 2mgMA group than the 1mgMA group, suggesting that a higher dose of MA reduces anxiety relative to a lower dose. While unexpected, it is consistent with the finding that rats treated with 2mgMA were not different in

anxiety-related behaviour, while the 1mgMA group produced higher anxiety than the saline only and naltrexone only groups.

At the one significant measure at PND100+, there was no significant difference in the percentage of changed arm entries and therefore indicates no difference in spatial WM behaviour between the 1mgMA and 2mgMA treated rats. Unexpectedly, this suggests that MA dose does not make a difference to behaviour. This outcome is in part consistent with the effect of each dose of MA on anxiety relative to the saline only and naltrexone only groups, except where MA may have improved WM performance.

b) Does MA Dose Alter the Effect of Naltrexone? There were no significant differences between N+1mgMA and N+2mgMA at any measure at either age, indicating that MA dose does not alter the effect of naltrexone. At EA, this outcome is not consistent with the effect of naltrexone whereby naltrexone was suggested to reduce the anxiety-inducing effect a lower dose, but not a higher dose of MA. It is also not consistent with the outcome that MA dose did make a difference to behaviour, as seen in Question 4a). It is also not consistent with the difference in the effect of each MA dose seen relative to baseline (saline only and naltrexone only groups).

At later adulthood, this outcome is consistent with the findings that naltrexone did not demonstrate an effect on either dose of MA. It is consistent with the finding that MA dose did not make a difference to behaviour, but inconsistent with the difference in effect of each dose of MA seen relative to baseline.

To summarise, from the presented results it may be inferred that a lower dose but not a higher dose of MA experienced during adolescence increases EA anxiety-related behaviour. At both doses MA did not produce a long-lasting spatial WM deficit in later adulthood. Rather, MA may lead to improved spatial WM in later adulthood. Naltrexone demonstrated a clear effect on a lower dose of MA at EA, suggesting anxiety-mitigating ability. However, at later adulthood, the results indicate that naltrexone demonstrated no enhancement of WM performance, and may actually impair spatial WM ability. In addition, the findings indicate that at no point in this experiment did MA impair spatial WM function and nor did naltrexone mitigate it at EA or, of interest, at later

adulthood. This may have been due to the measures used in the present experiment which differ from those traditionally used in studies of WM.

General Discussion

The results from the measures of anxiety and memory at each of the behavioural tests indicated that there were differences between groups in their behavioural outcomes, however generally not in the anticipated direction. That is, at EA and later adulthood, MA did not consistently induce higher anxiety-like behaviour or worse spatial WM performance. Naltrexone did not consistently attenuate these behaviours. These general outcomes were particularly evident at later adulthood for both MA and naltrexone, and most evident in the zero-maze test. The results for the other two tests (Y-maze and the light/dark box) were more complicated, and appeared more dependent on the age of testing, the behavioural measures in each test, and the dose of MA administered. A general summary of the of the effect of MA and naltrexone on anxiety-related behaviour is presented in the Table 4 below. The drugs did not demonstrate any anticipated effect on spatial WM behaviour in the Y-maze. This will be clarified later in this discussion.

Table 4

Summary of the Overall Interpretations of the Effect of MA and Naltrexone on Anxiety-Related Behaviour for All Three Tests at Both Testing Ages

	Did MA heighten anxiety?				Did Naltrexone mitigate anxiety?			
	1mg		2mg		1mg		2mg	
	LA	Adult	LA	Adult	LA	Adult	LA	Adult
Light/Dark Box	No	Ambiguous/ No	Yes	Ambiguous/ No	Ambiguous/ No	Ambiguous/ No	No	Yes
Zero Maze	No	No	No	No	No	No	No	No
Y maze	Yes	No	No	No	Yes	No	Yes	No

It should be noted that these conclusions are based on those significant measures at each test. The non-significant measures did not provide an indication of the effect of MA and naltrexone. However, this tabular summary describes the overall interpretation of the present findings with

respect to the two main aims of the research. The following is a discussion of whether these aims were met.

Aim 1: Does MA Experienced During Adolescence Have Long-Lasting Effects on the Rats' Behavioural Development?

In short, there is not enough evidence from the present findings to conclude that MA has a long-lasting effect on the development of anxiety and reduced spatial WM function. For no measure of memory (percentage of changed entries and observations in the Y-maze) at either testing age were there differences between groups illustrating that MA administration produced a reduction in spatial WM performance. This indicates that MA does not affect spatial WM. The expected anxiety-inducing effect of MA was only seen consistently with administration of a higher dose of MA in the light/dark box and of a lower dose of MA in the Y-maze, at EA. However, only one third of the possible results (one third of all measures) at EA indicated an anxiety-heightening effect of MA, and there was inconsistency in the effect of MA with respect to dose.

In later adulthood, neither dose of MA consistently heightened anxiety. This effect was difficult to determine from the results of two measures in the light dark box. Firstly, results from the measure 'observations in light' suggested that rats treated with 1mgMA demonstrated higher anxiety levels than rats treated with only naltrexone, indicating an anxiety-heightening effect of MA. However, 1mgMA treated rats did not demonstrate higher anxiety levels than rats treated with only saline, indicating no anxiety-heightening effect of MA. Secondly, as suggested by the results from the EL measure, rats treated with 2mgMA demonstrated higher anxiety levels than rats treated with only naltrexone or saline. However, the results from the observations in light measure suggest that rats treated with 2mgMA did not demonstrate higher anxiety levels than rats treated with only naltrexone or saline. These outcomes indicate that there was inconsistency in the effect of MA at this age group in the light-dark box. No results at the other two tests indicated that there was an anxiety-heightening effect of MA at later adulthood. Therefore, there was no clearly demonstrated long-lasting effect of either dose of MA on the rats' behavioural development in later adulthood.

Overall, there is insufficient evidence from the present results to support the idea that MA experienced during adolescence has long-lasting effects on the rats' behavioural development, particularly in later adulthood. This differs from prior claims that heightened anxiety and memory deficits are known outcomes of MA use (Teixeira-Gomes et al., 2015). However, there is an actual paucity of animal research on the long-term effect of adolescent-MA exposure on WM deficits and, in particular, on anxiety-related behaviour. The current findings are predominantly inconsistent with the theory, in the introduction of this study, which presented a mechanism by which MA would heighten anxiety and produce deficits in spatial WM.

Regarding WM, the present findings are also inconsistent with findings of the limited prior research. These prior findings illustrated a long-term decrease in WM performance in later adolescence, early adulthood (North et al., 2013) and later adulthood (Sherrill et al., 2013; Vorhees et al., 2005; Ye et al., 2014) after a period of adolescent MA-exposure. Vorhees et al. (2005) and North et al. (2013) demonstrated this effect on spatial WM using higher doses of MA (6.25mg/kg and 24mg/kg respectively), whilst Sherrill et al. (2013) and Ye et al. (2014) were not investigating specifically spatial WM. This could potentially explain the discrepancy between these findings with the findings from the present investigation.

Explanations of the Effect of MA on Anxiety and Spatial WM

There are multiple explanations for why at each test in the present investigation there was no consistent, clear anxiety-heightening or WM-impairing effect of MA demonstrated.

Firstly, the anxiety-enhancing effect of MA may have generally not been sustained across the age groups. This would explain the lack of anxiety effect of MA seen at later adulthood. The two tests where MA demonstrated an anxiety-inducing effect were seen at EA. Some literature has described the effect of MA abstinence as a process that, after use is discontinued, is known to involve emotional and cognitive impairments that can occur for months (Rawson, Gonzales, & Brethen, 2002). This is consistent with research by King and colleagues (2010) who demonstrated that human adolescents exhibited heightened anxiety (King et al., 2010b) and WM deficits (King et

al., 2010a) after being 4-11 months abstinent from MA. These findings align with the small effect seen at EA in the present study.

Other research also indicates that the effects of MA abstinence may not be prolonged for more than a few months. King et al. (2010a) demonstrated that the length of MA abstinence in these human adolescents was associated with improved performance on a digit span task- a test of WM capacity. Additionally, recent research by Yan et al. (2019) illustrated that seven consecutive days of 1mg/kg MA in adolescent rats (PND45-51) increased anxiety-related behaviour in the elevated plus maze and WM deficits in the Y-maze. These behaviours spontaneously recovered after long-term abstinence in later adulthood (Yan et al., 2019). These findings indicate that behaviours improve over time after the beginning of MA abstinence, which would support the interpretation of the present findings in later adulthood. Yet, there is still a lack of longitudinal research on MA, which is necessary to elucidate these long-term effects of MA in adolescents (King et al., 2010a).

Adolescent neuroprotection against MA could explain the present lack of effect of MA at EA, and particularly at later adulthood. The findings of previous research suggest that adolescent rodent brains are more protected from the neurotoxic effects of psychostimulants than adults, because the adolescent DA system is resistant to the effects of MA (Buck & Siegel, 2015; Teixeira-Gomes et al., 2015). Observationally, these findings have been with respect to the effect of higher doses of MA than in the present study, and they have not been with respect to behavioural outcomes.

Every measure used in the present study does not clearly reflect either anxiety or spatial WM, for various reasons. The following is an account of these measures, and the types of behaviour they may otherwise be reflecting.

The measures ‘total entries and ‘total observations in both arms’ in the Y-maze did not measure spatial WM. It is a recognition of a change in the novel arm in the Y-maze that reflects spatial WM (Hughes & Maginnity, 2007), however these two measures did not involve the rat demonstrating a preference for either arm. Therefore, they do not reflect a recognition of the

changed arm or the unchanged arm, or in turn, spatial WM. This impacts the interpretation of MA's (and subsequently naltrexone's) effect, as it is unclear what these measures are reflecting. For the purpose of the present investigation, the measures were treated as measures of anxiety, but any changes in these responses did not certainly reflect changes in anxiety.

The validity of 'percentage of light' and 'open entries' as measures of anxiety, each from the light-dark box and zero-maze respectively, is questionable, thereby affecting the interpretations of the effect of MA and naltrexone. These are ineffectual measures as there were only two options available for choice. The rat could either enter the light or dark compartments in the light/dark box, or the open or closed areas of the zero maze. For example, if a rat had entered the light compartment or open area 11 times, then it had also entered into the dark (or enclosed areas) 10 times (the rat started in the dark compartment/closed areas). Conversely, it would be 11 times if the rat ended the trial in the dark compartment or closed areas. Thus, the measures reflect that the rats had only a 50:50 choice, not a preference for the light compartment or the open areas. This explains the present results that all treatment groups demonstrated around 50 percent open entries in the zero maze (figure 8) and light entries in the Y-maze (figure 4). Therefore, it is questionable how these measures reflect anxiety-related behaviour in the present study.

There are other measures from the light-dark box and the zero-maze test that are also questionably less valid measures of anxiety than others. These also affect the interpretations that are made regarding the effect of MA and subsequently naltrexone. The light-dark box and zero maze tests are based on conflict of either approaching the 'curiosity-inducing' or avoiding the 'aversive' light compartment or open areas respectively. Approaching reflects lower anxiety while avoiding reflects higher anxiety ((Dixon & Hughes, 2019; Tucker & McCabe, 2017). However, the measures 'total entries of both the light/open and dark/closed areas' for these tests do not demonstrate that the rats had a preference for either compartment or area respectively. Thus, they cannot reflect a conflict between a curiosity to approach the light or anxiety leading to avoidance of the light by staying in the dark or enclosed area. Therefore, they are likely not reflecting anxiety-related behaviour.

All of these measures could reflect one of two options. Either anxiety, where fewer entries or observations reflect heightened anxiety and more entries or greater observation reflects lower anxiety. This is because it is known that lower levels of locomotion are associated with heightened anxiety (Archer, 1973; Belzung, 1999). Otherwise, these measures may be reflecting general motor activity due the nature of the tests which relies on the innate curiosity of rats to explore novel environments (Kraeuter, Guest, & Sarnyai, 2019). The seemingly more valid measures such as EL, observations in light/open areas (light/dark box and zero maze), and the percentage of changed entries and observations (Y-maze) are also reliant on the curiosity of the rats to explore and require motor activation. That is, all the measures were affected by the nature of the three tests used in the present study. Therefore, the inherent nature of some measures and the nature of each test impacts the validity of the measures and therefore confound the interpretation of the present results.

In addition to motor activation from the innate drive to explore new areas, potential motor stimulation effects from MA use may also confound the interpretations of the present results. However, for the purposes of the present study, results have not been interpreted with respect to motor-stimulation effects. Use of psychostimulants, such as MA, are known to stimulate motor activity (Asser & Taba, 2015), which is regulated by DA (Hayashizaki et al., 2013). Repeated exposure to a psychostimulant enhances this motor-stimulant response, an outcome known as behavioural sensitisation (Riday, Kosofsky, & Malanga, 2012; Steketee & Kalivas, 2011).

Motor-stimulation would be reflected in, for example, a greater total number of entries and observations in each testing apparatus. MA-induced motor activity may counteract the anxiety-inducing effects of MA, which could explain why MA was not seen to ‘heighten anxiety’ in the present findings, as illustrated in Table 4 above. That is, MA may still increase anxiety, however, the present tests are not clearly measuring only anxiety or WM, and the potential motor stimulation response to MA use may confound the results.

At measures where an anxiety-heightening effect of MA was seen may have been because the heightened anxiety effect over-rode the motor enhancing effect of MA. That is, during EA in the Y-maze for a 1mg dose of MA and in the light dark box for a 2mg dose of MA (see Table 4).

As well as possibly explaining the lack of MA-induced behavioural effects seen in this experiment, the potential confounding factors influencing the interpretations may also explain some of the paradoxical results found from the present investigation. In the light-dark box at later adulthood, N+2mgMA-treated rats were observed more often in the light than saline-treated rats. This could indicate an anxiolytic effect of MA which in turn could be a result of: the curiosity about and thus exploration of the novel light environment; possible attenuation of the anxiogenic effect of MA by naltrexone; the motor activity-inducing effect of MA, or a combination of all three. In addition, in the Y-maze at EA, rats treated with 2mgMA displayed higher total entries and total observations in both arms than 1mgMA treated rats. It would not make sense that a higher dose of MA would demonstrate lower anxiety than a lower dose of MA. Therefore, this finding may be reflecting an increase in motor activation with a higher dose of MA.

There were also paradoxical results with some of the seemingly more valid measures of anxiety and memory. In the Y-maze at later adulthood, 1mgMA and 2mgMA treated rats showed higher percentages of changed arm entries than naltrexone-only treated rats. Depending on the interpretation of this measure, this finding could indicate a variety of outcomes. That is, naltrexone had either a spatial WM-impairing or an anxiogenic effect, MA improved spatial WM or had an anxiolytic effect, or MA had a greater motor stimulant effect. In the zero maze at EA, N+2mgMA and 2mgMA treated rats had a significantly shorter EL than saline only treated rats, which indicates that a higher dose of MA was anxiolytic. It could otherwise be interpreted that the motor stimulant effect of MA lead to a shorter EL than saline. However, another potential reason for this finding in the zero maze is that there was a confounding factor that may have influenced the EL of the saline only group. At the PND60+ testing, the light controls to reduce the brightness were broken in the room the zero-maze apparatus was unable to be transferred to a different room. Subsequently, the lights were on high brightness, and it was essential to hover near the apparatus during testing as there was no CCTV set up to watch the rats via a television screen. This could very well have impacted the results by heightening anxiety levels in this saline group.

Aim 2: Can any Long-Term Effects of MA on Behaviour be Mitigated by Concurrent Exposure to Naltrexone?

The results indicate that for no measure of WM there were any differences between groups suggesting a mitigating effect of naltrexone at either age. Naltrexone's effect on anxiety was also minimal at both doses of MA, particularly at later adulthood. Consistent with discussing the results with respect to anxiety and WM (as opposed to motor activity), there are results of the present investigation regarding the effect of naltrexone for which there can be greater confidence. These results are based on the significant measures which, together, indicated a consistent anxiety-inducing effect of MA. Therefore, there can be greater confidence that the effect of naltrexone is one that is either mitigating or not mitigating the anxiety-inducing effect of MA at each measure.

The first outcome was at EA, where the results for both significant measures in the Y-maze suggested a consistent anxiety-reducing effect of naltrexone on a 1mg dose of MA. Additionally, there was no difference between the saline and naltrexone-only groups or the 1mgMA group at either measure, which may consistently indicate a strong anxiety-attenuating effect of naltrexone on a lower dose of MA. The second outcome was at also at EA, where the results of all significant measures at the light/dark box consistently suggested no mitigating effect of naltrexone on a 2mg dose of MA. While the results are clear for each measure, these two results illustrate an inconsistent effect of naltrexone. This may indicate that naltrexone is effective on a lower but not a higher dose of MA, at EA but not at later adulthood. This would suggest a lack of confidence in the attenuating effect of naltrexone.

This interpretation of the anxiety-mitigating effect of naltrexone seen in the Y-maze at EA was based on the two significant measures out of four in this test. As mentioned, 'total entries of and total observed in both arms' have not been suggested to measure spatial WM, as they do not reflect a preference for the novel arm. Rather, they are possibly measuring either general motor activity or anxiety. Based on the behavioural outcomes, these naltrexone results were interpreted in terms of anxiety. That is, at EA the reduced total entries and observations seen with a lower dose of MA likely reflects heightened anxiety, and the increased total entries and observations with

treatment of naltrexone on this lower dose likely reflects reduced anxiety. However, MA and naltrexone did not demonstrate these behavioural effects at a higher dose of MA or at later adulthood, indicating that the effect of naltrexone could just as likely be reflecting changes to anxiety or motor activity.

The interpretation that naltrexone demonstrated no effect on anxiety at EA in the light-dark box was based on the three significant measures out of the four measures at this test. In contrast to the Y-maze measures, one of these measures (total entries of both arms) was previously suggested to not be reflecting anxiety-related behaviour. The other two measures were acknowledged as likely more valid measures of anxiety (EL, observations in light). Again, naltrexone could therefore be affecting anxiety or motor activity. Overall, like MA, it remains uncertain whether the small effect of naltrexone seen is demonstrating changes in possible anxiety or motor stimulation effects of MA, affecting the present interpretations.

A particularly paradoxical result is where naltrexone demonstrated what was interpreted as an anxiety-reducing effect at EA on a higher dose of MA in the Y-maze- yet MA at this age and dose alone did not demonstrate an anxiety-heightening effect. This indicates the potential of naltrexone alone having anxiolytic properties, as opposed to only being anxiolytic when attenuating the anxiogenic effect of a drug like MA.

It may also be possible that at later adulthood the effect of naltrexone is reflecting an anxiety-mitigating effect, as illustrated in the light/dark box on a higher dose of MA (2mg). The measures reflecting this effect in the light/dark box were observations in light and EL, which both rely on a decision to move into the evasive area and may therefore more accurately reflect anxiety. However, the effect of MA was ambiguous. The 2mgMA group had a longer EL reflecting higher anxiety than the saline- and naltrexone-only groups, indicating that naltrexone was mitigating the effect of MA. In contrast, for the ‘observations in light’ measure, there were no significant differences between the 2mgMA group and the saline and naltrexone groups, which could indicate that naltrexone has an isolated anxiolytic effect of its own. This latter result is consistent with the paradoxical effect mentioned above. However, it is inconsistent with the outcome at this

‘observations in light’ measure as there was no difference in behaviour between the naltrexone-only and saline-only treated rats, which indicates no isolated anxiolytic effect of naltrexone. Based on the effect of MA at this 2mg dose and later adulthood, it is difficult to accurately determine what effect naltrexone is having on behaviour.

The findings regarding the effect of naltrexone (and MA) in the light dark box at later adulthood were more ambiguous when paired with a lower dose than a higher dose of MA. For ‘observations in light’ N+1mgMA treated rats were observed in the light more often than 1mgMA (and 2mgMA) treated rats. This indicates an anxiety-mitigating effect of naltrexone. However, there was inconsistency in the effect of MA at this measure. The saline group was not significantly different to 1mgMA treated rats, indicating no difference in anxiety. However, 1mgMA treated rats were observed in the light significantly less often than the naltrexone group, indicating that 1mgMA rats exhibited higher anxiety.

In contrast to the mitigating effect of naltrexone indicated from the ‘observations in light’ measure, results from the EL measure indicate that naltrexone had no effect on anxiety-related behaviour. That is, there were no significant differences between the N+1mgMA or the 1mgMA groups. Results for EL also indicated that MA had no effect on anxiety-related behaviour, suggested by the outcome that the 1mgMA group was not significantly different to the saline and naltrexone treated rats. Again, this contrasts with the inconsistent effect of MA seen from the ‘observations in light’ measure. Together, these results illustrate an inconsistent and unclear effect of naltrexone on a 1mg dose of MA at later adulthood.

Explanations of the Effect of Naltrexone on Anxiety and Spatial WM

The overall lacking long-term effect of MA may interfere with the interpretations of naltrexone’s effect, making it hard to determine naltrexone’s true attenuating action from the present investigation. That is, where naltrexone did not demonstrate a mitigating effect, it may be interpreted that naltrexone is an ineffective pharmacotherapeutic agent in the treatment of MA-induced developmental effects at any later age. However, it may be more reasonable to suggest that

naltrexone's apparent ineffectiveness was because generally MA did not heighten anxiety or impair spatial WM. Naltrexone was intended to mitigate MA-induced behaviour and was not expected to have anxiolytic properties or improve spatial WM by itself. Some findings suggested that naltrexone may have stand-alone anxiolytic action. However, naltrexone-treated rats generally did not demonstrate anxiety levels different to those treated with only saline, indicating that naltrexone does not have isolated anxiolytic properties. The minimal anxiety-reducing and lack of WM-enhancing effect seen from naltrexone is therefore unsurprising.

The present effect of naltrexone may otherwise reflect the complex involvement of various neurotransmitter systems in the behavioural effect of MA. Firstly, MA has demonstrated agonistic action at opioid receptors (Chiu et al., 2006) and indirect agonistic DA action via the opioid system (Schad et al., 2002). Naltrexone is a known non-selective opioid antagonist (Dimatelis et al., 2012). Research by Jayaram-Lindstrom et al. (2017) has alluded to naltrexone's indirect action on MA-induced physiological effects, demonstrating indirect DA antagonism of amphetamine-induced DA agonism. MA's action on the DA system is also known as the predominant mechanism of MA-induced pharmacological and behavioural effects (Ballester et al., 2017; Nickell et al., 2014). Therefore, more direct DA antagonism may produce different behavioural effects from naltrexone.

Secondly, though less studied, it is known that MA acts via various other hormones and neurotransmitters. For example, in addition to primary DA action, MA acts via other monoamine neurotransmitters (Ballester et al., 2017; Nickell et al., 2014). These include serotonin and adrenaline, for example. Opioids, GABA, glutamate, ACh and some stress hormones are also implicated in mediating the acute and chronic effects of MA use (Ballester et al., 2017). Along with the secondary involvement of these neurotransmitters and hormones, MA-induces interactions of these systems with DA systems (Moratalla et al., 2017).

Together, these findings illustrate that due to the complex action of MA, treatment of MA should likely be targeting multiple neurotransmitter systems, which naltrexone does not. This illustrates the likely value of investigating these less studied neurotransmitter systems to determine potential treatment targets other than, or as well as naltrexone.

Differential Effect of Sex on Behaviour

While the effects of MA and naltrexone remain inconclusive, outcomes of the present study demonstrated behavioural differences between male and female rats. There were no interaction effects, indicating that the sex of the rat did not influence the effect of MA and naltrexone on anxiety and spatial WM behaviour. However, there was an overall difference between males and females with respect to anxiety-related responses. Males and females were not different on every measure at each testing age, however, where there was a significant difference between them, male rats consistently displayed higher anxiety-related behaviour than female rats. This indicates that males are more susceptible to heightened anxiety than females, highlighting that sex may be a confound to interpreting results in research that has used either only males or females.

Limitations and Future Directions

All of the measures in each test limited the interpretations of the present investigation. The question remains whether the behaviours observed truly reflected anxiety and memory or otherwise, such as motor activity. This is for two main reasons. Firstly, due to the subjects used, particularly anxiety as a subjective concept had to be inferred from the rats' behaviours as they cannot describe how they are feeling as per humans. Spatial WM performance was also inferred from each rat's behaviour. Secondly as discussed, the ethological nature of each test in the present study relied on the innate curiosities of the rats to explore the novel environments (Kraeuter et al., 2019). While a strength of this is that prior learning and reinforcers are not required, general motor activity became a confound to interpreting the results. This is in addition to the potential motor stimulating effects of repeated MA use (Asser & Taba, 2015; Riday et al., 2012; Steketee & Kalivas, 2011).

This limitation was exacerbated by the measures with more questionable validity which were more likely not reflecting anxiety. The 'total entries of both' measures in the light dark box and zero maze did not inherently reflect a preference for any part of each testing apparatus. The 'percentage of light' and 'open entries' measures gave no clear indication of anxiety-related behaviour with the outcome being consistently close to 50% for both measures. These factors interfere with and limit the conclusions that can be made with respect to the effect of MA and naltrexone. Additionally, and likely due to these factors, under half of the total measures used from all three tests were significant, further limiting conclusions that can be made and the confidence in the present outcomes. In the future, using tests that minimise this confound of motor activity and use of more valid measures of anxiety and memory behaviours should more clearly and accurately reflect the long-term effects of MA and naltrexone.

The present research investigated mitigating MA-induced effects preventatively by administering a drug (naltrexone) prior to MA administration. Consequently, the interpretation of naltrexone's effect was found in, and applicable to, the context of administering a DA antagonist prior to every dose of MA. However, this may not be very realistic for a human situation. While

possible, the likelihood of adolescents having [naltrexone] treatment immediately prior to both their first administration of MA and subsequent consecutive MA administrations seems relatively slim. It is more realistic that by the time a DA antagonist is administered, repeated or chronic MA use may have produced, for example, DA hypofunction (Ballester et al., 2017) through neurodegeneration (Luikinga et al., 2018). This long-term antagonistic action of MA would require agonistic treatment as opposed to the antagonistic action produced by naltrexone. For example, Williams and Castner (2006) illustrated that both insufficient (as per chronic MA administration) and excessive (as per acute MA administration) prefrontal DA produce WM impairments. These impairments were reduced by treatment with a DA receptor agonist and antagonist, respectively (Williams & Castner, 2006). This necessitates treatment for both contexts; prior to and after repeated MA use.

Another potential issue was that there was no adult comparison. That is, to determine if adolescents are more vulnerable to the long-term behavioural effects of repeated MA exposure, direct statistical comparison to adult MA-exposed rats is necessary. Consistent demonstration that adolescent exposure to MA produces worse later adulthood anxiety and memory performance than adult exposure to MA would infer a need for tailored treatment for adolescents. However, even if there are no behavioural differences between adolescents and adults, adolescents may need to be treated differently regardless due to brain changes occurring during adolescent development (Arain et al., 2013). The research gap investigating adolescent-MA effects (Luikinga et al., 2018) and the paucity of studies comparing the effects of MA at adolescence and adulthood necessitates a clear focus for future research. That is, further investigation of the long-term behavioural and physiological effects from adolescent exposure to MA, and investigation of the potential differences in MA-induced effects between adolescents and adults. This will provide further insight into treatment targets.

Psychostimulant use is known to start predominantly during adolescence (Luikinga et al., 2018), inferring that MA-initiation could occur at any stage of adolescent development. Yet, the present study was investigating only a snippet of the whole adolescent period, during

periadolescence from PND41-50 (Vorhees et al., 2005). Literature has indicated that between as early as PND21 until as late as PND60 constitutes adolescence in rodents (Laviola, Macrì, Morley-Fletcher, & Adriani, 2003; Marco et al., 2011). Additionally, there is currently insufficient research on the long-term effects of adolescent MA-exposure to be conclusive on the exact age (PND) at which most suggested heightened vulnerability is seen. Therefore, as well as a direct comparison to adults, direct comparison of the peri-adolescent stage should be made with other stages of adolescent development. This will help to determine if and where during adolescence there exists a period with a greater vulnerability to the long-term effects of MA than other stages of adolescence.

As mentioned, more direct DA targeting and targeting of other neurotransmitter systems involved in MA effects in relevant brain regions will enhance research insight into treatment targets. This is due to the primary involvement of DA, the secondary involvement of other neurotransmitter systems (Ballester et al., 2017), and the complex interactions of the DA system with these other neurotransmitter systems (Moratalla et al., 2017; Zarrindast & Khakpai, 2015). This should involve both the separate and combined investigation of drugs impacting the multiple MA-implicated hormones and neurotransmitter systems. In turn, this will allow the targets which demonstrate the most potential in mitigating MA-induced anxiety and impaired spatial WM performance to be determined.

The present study was initially designed to investigate the effect of two drugs; naltrexone and lobeline, which may have differing physiological effects on MA-implicated neurotransmitter systems. However due the inaccessibility of obtaining lobeline, effects of this drug were unable to be investigated in the present study. The rationale for the use of lobeline was its physiological potential as a pharmacotherapeutic agent in the treatment of MA dependence (Dimatelis et al., 2012; Nickell et al., 2014). This is due to the overlap in MA and lobeline's physiological actions on neurotransmitter systems in the brain. The main action of MA is predominantly via its effects on the vesicular monoamine transporter 2 (VMAT2), reversing its function to increase cytosolic DA levels (Morley et al., 2017). This action, along with subsequent cytosolic monoamine oxidase (MAO) inhibition, creates a pool of available DA for transport to postsynaptic DA receptors (Dwoskin &

Crooks, 2002) via MA-induced reversed DA transporter (DAT) function (Taylor et al., 2013). Therefore MA produces post-synaptic DA agonistic action (Cruickshank & Dyer, 2009).

Lobeline functions to inhibit VMAT2, but in comparison to MA, resultant cytosolic DA is broken down by MAO (Dwoskin & Crooks, 2002). Administered together, lobeline prevents the MA-induced formation of an available pool of DA, thus acting as an indirect DA receptor antagonist (Dwoskin & Crooks, 2002). Potentially the presence of lobeline competes with MA function, allowing more DA to be exposed to MAO metabolism therefore attenuating MA physiological effects. Lobeline has also been seen to function as a DAT inhibitor (Wilhelm, Johnson, Eshleman, & Janowsky, 2008), and as an antagonist at (mu)opioid receptors (as per naltrexone) (Dennis K. Miller et al., 2007) and at nicotinic receptors (Dennis K. Miller, Crooks, & Dwoskin, 2000). It has also been shown to selectively inhibit the effect of amphetamines (D. K. Miller et al., 2001). Therefore, lobeline acts on both primary and secondary targets of MA.

Results of mouse and clinical studies suggest that VMAT2 inhibition might reduce behaviours induced by MA (Nickell et al., 2014). For example, lobeline has been seen to attenuate amphetamine-induced LA and the drug's discriminative stimulus properties (D. K. Miller et al., 2001), as well as decrease MA self-administration (Harrod, Dwoskin, Crooks, Klebaur, & Bardo, 2001). Therefore, through both its direct DA action and effect on secondary MA-implicated neurotransmitter systems, lobeline presents as a treatment option for potential adolescent MA-induced anxiety and WM impairments that is worthy of future investigation.

Finally, there is a predominance of neuroscience and biomedical studies only using male animals in their research (Beery & Zucker, 2011). The present findings that male and female rodents differ behaviourally highlights that extrapolating male-only data to female populations poses a potential problem in making accurate research interpretations. It is pertinent that this potential confound is acknowledged by including and accounting for the effect of both males and females in future animal research.

Conclusions

The present research aimed to address the research gap investigating the long-term behavioural effects of adolescent MA use (Luikinga et al., 2018). Collectively, prior research has also suggested that adolescence is a period of heightened vulnerability to the potential long-term anxiogenic and impaired spatial WM effects of MA. Naltrexone as an opioid receptor antagonist (Dimatelis et al., 2012) has a suggested capability to mitigate the physiological action of MA, and therefore the potential behavioural effects induced from MA use. This provided a rationale for the present research.

Contrary to expectation, adolescent exposure to MA did not demonstrate a clear overall anxiety-heightening and spatial WM-impairing effect in later adulthood. Naltrexone was not expected to produce an isolated anxiolytic effect and therefore did not nor could not demonstrate a clear reduction in the deleterious long-term effects of MA. Outcomes of these two drugs were difficult to interpret, particularly in the Y-maze and light/dark box. This is because the effect of the drugs on behaviour was inconsistent and complex, where the effects appeared dependent on a variety of factors such as; age, the behavioural measures and MA dose. In contrast to the unclear findings on the effect of MA and naltrexone, male rats consistently demonstrated heightened anxiety relative to female rats at both EA and later adulthood.

There is insufficient evidence from the present findings to conclude that adolescent exposure to MA leads to the development of heightened anxiety or, particularly, reduced spatial WM performance in later adulthood. It can also not be concluded that adolescent treatment with naltrexone mitigates the development of MA-induced effects in later adulthood. The present investigation highlights potential reasons for this effect, including limitations in the methodology of this research that could be addressed in future research to more clearly and accurately elucidate the current findings. Research should also note the behavioural difference found between males and females and more cautiously take this into account when interpreting pharmacological effects in future investigations.

Irrespective of these outcomes, there is still an obvious need for investigative research into the long-term behavioural MA effects from repeated adolescent use. In addition, there is a need for further research into pharmacotherapeutic options due to the current lack of effective or approved pharmacological agents in MA treatment (Courtney & Ray, 2014; Petit et al., 2012). This research area would benefit from a focus on treatment agents targeting various MA-implicated neurotransmitters, hormones and brain regions that are developing during the different stages of adolescence. This will assist in identifying the brain physiology and anatomy and stage of adolescence that is most vulnerable to the deleterious long-term effects of MA use.

References

Andersen, S. L. (2003). Trajectories of Brain Development: Point of Vulnerability or Window of Opportunity? *Neuroscience and Biobehavioral Reviews, 27*(1-2), 3-18.

Arain, M., Haque, M., Johal, L., Mathur, P., Nel, W., Rais, A., . . . Sharma, S. (2013). Maturation of the Adolescent Brain. *Neuropsychiatric Disease and Treatment, 9,* 449-461. doi:10.2147/ndt.s39776

Archer, J. (1973). Tests for Emotionality in Rats and Mice: A Review. *Animal Behaviour, 21*(2), 205-235.

Asser, A., & Taba, P. (2015). Psychostimulants and Movement Disorders. *Frontiers in Neurology, 6*, 75. doi:10.3389/fneur.2015.00075

Balcells-Olivero, M., & Vezina, P. (1997). Effects of Naltrexone on Amphetamine-Induced Locomotion and Rearing: Acute and Repeated Injections. *Psychopharmacology, 131*(3), 230-238.

Ballester, J., Valentine, G., & Sofuoglu, M. (2017). Pharmacological Treatments for Methamphetamine Addiction: Current Status and Future Directions. *Expert Review of Clinical Pharmacology, 10*(3), 305-314. doi:10.1080/17512433.2017.1268916

Beery, A. K., & Zucker, I. (2011). Sex Bias in Neuroscience and Biomedical Research. *Neuroscience and Biobehavioral Reviews, 35*(3), 565-572. doi:10.1016/j.neubiorev.2010.07.002

Belzung, C. (1999). Chapter 4.11 Measuring Rodent Exploratory Behavior. *Techniques in the Behavioral and Neural Sciences, 13*(C), 738-749. doi:10.1016/S0921-0709(99)80057-1

Bentley, J., Heard, J., Collins, G., & Chung, C. (2015). Mixing Medicines: How to Ensure Patient Safety. *Pharmaceutical Journal, 294*(7859), 453-456.

Bourque, M., Liu, B., Dluzen, D. E., & Di Paolo, T. (2011). Sex Differences in Methamphetamine Toxicity in Mice: Effect on Brain Dopamine Signaling Pathways. *Psychoneuroendocrinology, 36*(7), 955-969. doi:10.1016/j.psyneuen.2010.12.007

Buck, J. M., & Siegel, J. A. (2015). The Effects of Adolescent Methamphetamine Exposure. *Frontiers in Neuroscience, 9,* 151. doi:10.3389/fnins.2015.00151

Casey, B. J., Jones, R. M., & Hare, T. A. (2008). The Adolescent Brain. *Annals of the New York Academy of Sciences, 1124,* 111-126. doi:10.1196/annals.1440.010

Chiu, C. T., Ma, T., & Ho, I. K. (2005). Attenuation of Methamphetamine-Induced Behavioral Sensitization in Mice by Systemic Administration of Naltrexone. *Brain Research Bulletin, 67*(1), 100-109. doi:10.1016/j.brainresbull.2005.05.028

Chiu, C. T., Ma, T., & Ho, I. K. (2006). Methamphetamine-Induced Behavioral Sensitization in Mice: Alterations in Mu-Opioid Receptor. *Journal of Biomedical Science, 13*(6), 797-811. doi:10.1007/s11373-006-9102-x

Corcoran, L., Roche, M., & Finn, D. P. (2015). The Role of the Brain's Endocannabinoid System in Pain and Its Modulation by Stress. *International Review of Neurobiology, 125,* 203-255. doi:10.1016/bs.irn.2015.10.003

Courtney, K. E., & Ray, L. A. (2014). Methamphetamine: An Update on Epidemiology, Pharmacology, Clinical Phenomenology, and Treatment Literature. *Drug and Alcohol Dependence, 143,* 11-21. doi:10.1016/j.drugalcdep.2014.08.003

Cruickshank, C. C., & Dyer, K. R. (2009). A Review of the Clinical Pharmacology of Methamphetamine. *Addiction, 104*(7), 1085-1099. doi:10.1111/j.1360-0443.2009.02564.x

Dimatelis, J. J., Russell, V. A., Stein, D. J., & Daniels, W. M. (2012). The Effects of Lobeline and Naltrexone on Methamphetamine-Induced Place Preference and Striatal Dopamine and Serotonin Levels in Adolescent Rats with a History of Maternal Separation. *Metabolic Brain Disease, 27*(3), 351-361. doi:10.1007/s11011-012-9288-8

Dixon, E. I., & Hughes, R. N. (2019). Treatment with 1-Benzylpiperazine (Bzp) During Adolescence of Male and Female Hooded Rats Exposed to Environmental Enrichment: Subsequent Behavioral Outcomes. *International Journal of Developmental Neuroscience 73,* 32-40. doi:10.1016/j.ijdevneu.2018.12.005

Dluzen, D. E., & Liu, B. (2008). Gender Differences in Methamphetamine Use and Responses: A Review. *Gender Medicine, 5*(1), 24-35.

Dwoskin, L. P., & Crooks, P. A. (2002). A Novel Mechanism of Action and Potential Use for Lobeline as a Treatment for Psychostimulant Abuse. *Biochemical Pharmacology, 63*(2), 89-98.

Ford, C. P., Mark, G. P., & Williams, J. T. (2006). Properties and Opioid Inhibition of Mesolimbic Dopamine Neurons Vary According to Target Location. *The Journal of Neuroscience, 26*(10), 2788-2797. doi:10.1523/JNEUROSCI.4331-05.2006

Georgiou, P., Zanos, P., Garcia-Carmona, J. A., Hourani, S., Kitchen, I., Laorden, M. L., & Bailey, A. (2016). Methamphetamine Abstinence Induces Changes in Mu-Opioid Receptor, Oxytocin and Crf Systems: Association with an Anxiogenic Phenotype. *Neuropharmacology, 105,* 520-532. doi:10.1016/j.neuropharm.2016.02.012

Guo, L. K., Wang, Z. Y., Lu, G. Y., Wu, N., Dong, G. M., Ma, C. M., . . . Li, J. (2019). Inhibition of Naltrexone on Relapse in Methamphetamine Self-Administration and Conditioned Place Preference in Rats. *European Journal of Pharmacology, 865,* 172671. doi:10.1016/j.ejphar.2019.172671

Haggkvist, J., Bjorkholm, C., Steensland, P., Lindholm, S., Franck, J., & Schilstrom, B. (2011). Naltrexone Attenuates Amphetamine-Induced Locomotor Sensitization in the Rat. *Addiction Biology, 16*(1), 20-29. doi:10.1111/j.1369-1600.2009.00199.x

Harrod, S. B., Dwoskin, L. P., Crooks, P. A., Klebaur, J. E., & Bardo, M. T. (2001). Lobeline Attenuates D-Methamphetamine Self-Administration in Rats. *The Journal of Pharmacology and Experimental Therapeutics, 298*(1), 172-179.

Hascoet, M., Bourin, M., & Nic Dhonnchadha, B. A. (2001). The Mouse Light-Dark Paradigm: A Review. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 25*(1), 141-166.

Hayashizaki, S., Hirai, S., Ito, Y., Honda, Y., Arime, Y., Sora, I., . . . Takada, M. (2013). Methamphetamine Increases Locomotion and Dopamine Transporter Activity in Dopamine D5 Receptor-Deficient Mice. *PLoS One, 8*(10), e75975. doi:10.1371/journal.pone.0075975

Hellem, T. L. (2016). A Review of Methamphetamine Dependence and Withdrawal Treatment: A Focus on Anxiety Outcomes. *Journal of Substance Abuse Treatment, 71*, 16-22. doi:10.1016/j.jsat.2016.08.011

Hughes, R. N., & Maginnity, M. E. (2007). Cues Used by Male and Female Hooded Rats for Locating a Brightness Change. *Behavioural Processes, 74*(1), 79-87. doi:10.1016/j.beproc.2006.09.014

Jayaram-Lindstrom, N., Guterstam, J., Haggkvist, J., Ericson, M., Malmlof, T., Schilstrom, B., . . . Franck, J. (2017). Naltrexone Modulates Dopamine Release Following Chronic, but Not Acute Amphetamine Administration: A Translational Study. *Translational Psychiatry, 7*(4), e1104. doi:10.1038/tp.2017.79

Johansen, A., & McFadden, L. M. (2017). The Neurochemical Consequences of Methamphetamine Self-Administration in Male and Female Rats. *Drug and Alcohol Dependence, 178*, 70-74. doi:10.1016/j.drugalcdep.2017.04.011

Johnson, S. W., & North, R. A. (1992). Opioids Excite Dopamine Neurons by Hyperpolarization of Local Interneurons. *The Journal of Neuroscience, 12*(2), 483-488.

King, G., Alicata, D., Cloak, C., & Chang, L. (2010a). Neuropsychological Deficits in Adolescent Methamphetamine Abusers. *Psychopharmacology, 212*(2), 243-249. doi:10.1007/s00213-010-1949-x

King, G., Alicata, D., Cloak, C., & Chang, L. (2010b). Psychiatric Symptoms and Hpa Axis Function in Adolescent Methamphetamine Users. *Journal of Neuroimmune Pharmacology, 5*(4), 582-591. doi:10.1007/s11481-010-9206-y

Kohno, M., Dennis, L. E., McCready, H., Schwartz, D. L., Hoffman, W. F., & Korthuis, P. T. (2018). A Preliminary Randomized Clinical Trial of Naltrexone Reduces Striatal Resting State Functional Connectivity in People with Methamphetamine Use Disorder. *Drug and Alcohol Dependence, 192*, 186-192. doi:10.1016/j.drugalcdep.2018.07.045

Konrad, K., Firk, C., & Uhlhaas, P. J. (2013). Brain Development During Adolescence: Neuroscientific Insights into This Developmental Period. *Deutsches Arzteblatt International, 110*(25), 425-431. doi:10.3238/arztebl.2013.0425

Kraeuter, A.-K., Guest, P. C., & Sarnyai, Z. (2019). The Y-Maze for Assessment of Spatial Working and Reference Memory in Mice. *Methods in Molecular Biology 1916*, 105.

Laviola, G., Macrì, S., Morley-Fletcher, S., & Adriani, W. (2003). Risk-Taking Behavior in Adolescent Mice: Psychobiological Determinants and Early Epigenetic Influence. *Neuroscience and Biobehavioral Reviews, 27*(1), 19-31. doi:10.1016/S0149-7634(03)00006-X

Lee, K. W., Kim, H. C., Lee, S. Y., & Jang, C. G. (2011). Methamphetamine-Sensitized Mice Are Accompanied by Memory Impairment and Reduction of N-Methyl-D-Aspartate Receptor Ligand Binding in the Prefrontal Cortex and Hippocampus. *Neuroscience, 178*, 101-107. doi:10.1016/j.neuroscience.2011.01.025

Luikinga, S. J., Kim, J. H., & Perry, C. J. (2018). Developmental Perspectives on Methamphetamine Abuse: Exploring Adolescent Vulnerabilities on Brain and Behavior. *Progress in Neuropsychopharmacology & Biological Psychiatry, 87*(Part A), 78-84. doi:10.1016/j.pnpbp.2017.11.010

Mancino, M. J., Gentry, B. W., Feldman, Z., Mendelson, J., & Oliveto, A. (2011). Characterizing Methamphetamine Withdrawal in Recently Abstinent Methamphetamine Users: A Pilot Field Study. *The American Journal of Drug and Alcohol Abuse, 37*(2), 131-136. doi:10.3109/00952990.2010.543998

Marco, E. M., Adriani, W., Ruocco, L. A., Canese, R., Sadile, A. G., & Laviola, G. (2011). Neurobehavioral Adaptations to Methylphenidate: The Issue of Early Adolescent Exposure. *Neuroscience and Biobehavioral Reviews, 35*(8), 1722-1739. doi:10.1016/j.neubiorev.2011.02.011

Meilandt, W. J., Barea-Rodriguez, E., Harvey, S. A., & Martinez, J. L., Jr. (2004). Role of Hippocampal Ca3 Mu-Opioid Receptors in Spatial Learning and Memory. *The Journal of Neuroscience, 24*(12), 2953-2962. doi:10.1523/jneurosci.5569-03.2004

Miller, D. K., Crooks, P. A., & Dwoskin, L. P. (2000). Lobeline Inhibits Nicotine-Evoked [3h]Dopamine Overflow from Rat Striatal Slices and Nicotine-Evoked 86rb + Efflux from Thalamic Synaptosomes. *Neuropharmacology, 39*(13), 2654-2662. doi:10.1016/S0028-3908(00)00140-4

Miller, D. K., Crooks, P. A., Teng, L., Witkin, J. M., Munzar, P., Goldberg, S. R., . . . Dwoskin, L. P. (2001). Lobeline Inhibits the Neurochemical and Behavioral Effects of Amphetamine. *Journal of Pharmacology and Experimental Therapeutics, 296*(3), 1023-1034.

Miller, D. K., Lever, J. R., Rodvelt, K. R., Baskett, J. A., Will, M. J., & Kracke, G. R. (2007). Lobeline, a Potential Pharmacotherapy for Drug Addiction, Binds to Mu Opioid Receptors and Diminishes the Effects of Opioid Receptor Agonists. *Drug and Alcohol Dependence, 89*(2-3), 282.

Mizoguchi, K., Yuzurihara, M., Ishige, A., Sasaki, H., Chui, D. H., & Tabira, T. (2000). Chronic Stress Induces Impairment of Spatial Working Memory Because of Prefrontal Dopaminergic Dysfunction. *The Journal of Neuroscience, 20*(4), 1568-1574.

Moratalla, R., Khairnar, A., Simola, N., Granado, N., García-Montes, J. R., Porceddu, P. F., . . . Morelli, M. (2017). Amphetamine-Related Drugs Neurotoxicity in Humans and in Experimental Animals: Main Mechanisms. *Progress in Neurobiology, 155*, 149-170. doi:10.1016/j.pneurobio.2015.09.011

Morley, K. C., Cornish, J. L., Faingold, A., Wood, K., & Haber, P. S. (2017). Pharmacotherapeutic Agents in the Treatment of Methamphetamine Dependence. *Expert Opinion on Investigational Drugs, 26*(5), 563-578. doi:10.1080/13543784.2017.1313229

Nagai, T., Takuma, K., Dohniwa, M., Ibi, D., Mizoguchi, H., Kamei, H., . . . Yamada, K. (2007). Repeated Methamphetamine Treatment Impairs Spatial Working Memory in Rats: Reversal

by Clozapine but Not Haloperidol. *Psychopharmacology, 194*(1), 21-32. doi:10.1007/s00213-007-0820-1

Nickell, J. R., Siripurapu, K. B., Vartak, A., Crooks, P. A., & Dwoskin, L. P. (2014). The Vesicular Monoamine Transporter-2: An Important Pharmacological Target for the Discovery of Novel Therapeutics to Treat Methamphetamine Abuse. *Advances in Pharmacology, 69*, 71-106. doi:10.1016/b978-0-12-420118-7.00002-0

North, A., Swant, J., Salvatore, M. F., Gamble-george, J., Prins, P., Butler, B., . . . Khoshbouei, H. (2013). Chronic Methamphetamine Exposure Produces a Delayed, Long-Lasting Memory Deficit. *Synapse, 67*(5), 245-257. doi:10.1002/syn.21635

O'Shea, M., Singh, M. E., McGregor, I. S., & Mallet, P. E. (2004). Chronic Cannabinoid Exposure Produces Lasting Memory Impairment and Increased Anxiety in Adolescent but Not Adult Rats. *Journal of Psychopharmacology, 18*(4), 502-508. doi:10.1177/026988110401800407

Okita, K., Ghahremani, D. G., Payer, D. E., Robertson, C. L., Dean, A. C., Mandelkern, M. A., & London, E. D. (2016). Emotion Dysregulation and Amygdala Dopamine D2-Type Receptor Availability in Methamphetamine Users. *Drug and Alcohol Dependence, 161*, 163-170. doi:10.1016/j.drugalcdep.2016.01.029

Olive, M. F., Koenig, H. N., Nannini, M. A., & Hodge, C. W. (2001). Stimulation of Endorphin Neurotransmission in the Nucleus Accumbens by Ethanol, Cocaine, and Amphetamine. *The Journal of Neuroscience, 21*(23), Rc184.

Orikabe, L., Yamasue, H., Inoue, H., Takayanagi, Y., Mozue, Y., Sudo, Y., . . . Kasai, K. (2011). Reduced Amygdala and Hippocampal Volumes in Patients with Methamphetamine Psychosis. *Schizophrenia Research, 132*(2-3), 183-189. doi:10.1016/j.schres.2011.07.006

Panenka, W. J., Procyshyn, R. M., Lecomte, T., MacEwan, G. W., Flynn, S. W., Honer, W. G., & Barr, A. M. (2013). Methamphetamine Use: A Comprehensive Review of Molecular, Preclinical and Clinical Findings. *Drug and Alcohol Dependence, 129*(3), 167-179. doi:10.1016/j.drugalcdep.2012.11.016

Peterson, E. K., & Hughes, R. N. (2017). Some Observations of Anxiety-Related Behavior in Young Adult Rats Following Late Adolescent Exposure to Methamphetamine with and without Environmental Enrichment. *Current Psychopharmacology, 6*(2), 172-178.

Petit, A., Karila, L., Chalmin, F., & Lejoyeux, M. (2012). Methamphetamine Addiction: A Review of the Literature. *Journal of Addiction Research & Therapy, S1*, 1-6. doi:10.4172/2155-6105.S1-006

Randall-Thompson, J. F., Pescatore, K. A., & Unterwald, E. M. (2010). A Role for Delta Opioid Receptors in the Central Nucleus of the Amygdala in Anxiety-Like Behaviors. *Psychopharmacology, 212*(4), 585-595. doi:10.1007/s00213-010-1980-y

Rawson, R. A., Gonzales, R., & Brethen, P. (2002). Treatment of Methamphetamine Use Disorders: An Update. *Journal of Substance Abuse Treatment, 23*(2), 145-150. doi:10.1016/S0740-5472(02)00256-8

Riday, T. T., Kosofsky, B. E., & Malanga, C. J. (2012). The Rewarding and Locomotor-Sensitizing Effects of Repeated Cocaine Administration Are Distinct and Separable in Mice. *Neuropharmacology, 62*(4), 1858-1866. doi:10.1016/j.neuropharm.2011.12.011

Sarnyai, Z., Sibille, E. L., Pavlides, C., Fenster, R. J., McEwen, B. S., & Toth, M. (2000). Impaired Hippocampal-Dependent Learning and Functional Abnormalities in the Hippocampus in Mice Lacking Serotonin(1a) Receptors. *Proceedings of the National Academy of Sciences of the United States of America, 97*(26), 14731-14736. doi:10.1073/pnas.97.26.14731

Schad, C. A., Justice, J. B., Jr., & Holtzman, S. G. (2002). Endogenous Opioids in Dopaminergic Cell Body Regions Modulate Amphetamine-Induced Increases in Extracellular Dopamine Levels in the Terminal Regions. *The Journal of Pharmacology and Experimental Therapeutics, 300*(3), 932-938.

Schindler, C. W., Bross, J. G., & Thorndike, E. B. (2002). Gender Differences in the Behavioral Effects of Methamphetamine. *European Journal of Pharmacology, 442*(3), 231-235.

Schramm-Sapyta, N. L., Walker, Q. D., Caster, J. M., Levin, E. D., & Kuhn, C. M. (2009). Are Adolescents More Vulnerable to Drug Addiction Than Adults? Evidence from Animal Models. *Psychopharmacology, 206*(1), 1-21. doi:10.1007/s00213-009-1585-5

Shepherd, J. K., Grewal, S. S., Fletcher, A., Bill, D. J., & Dourish, C. T. (1994). Behavioural and Pharmacological Characterisation of the Elevated "Zero-Maze" as an Animal Model of Anxiety. *Psychopharmacology, 116*(1), 56-64.

Sherrill, L. K., Stanis, J. J., & Gulley, J. M. (2013). Age-Dependent Effects of Repeated Amphetamine Exposure on Working Memory in Rats. *Behavioural Brain Research, 242*, 84-94. doi:10.1016/j.bbr.2012.12.044

Solati, J., Zarrindast, M. R., & Salari, A. A. (2010). Dorsal Hippocampal Opioidergic System Modulates Anxiety-Like Behaviors in Adult Male Wistar Rats. *Psychiatry and Clinical Neurosciences, 64*(6), 634-641. doi:10.1111/j.1440-1819.2010.02143.x

Spear, L. P. (2000). The Adolescent Brain and Age-Related Behavioral Manifestations. *Neuroscience and Biobehavioral Reviews, 24*(4), 417-463. doi:10.1016/S0149-7634(00)00014-2

Steketee, J. D., & Kalivas, P. W. (2011). Drug Wanting: Behavioral Sensitization and Relapse to Drug-Seeking Behavior. *Pharmacological Reviews, 63*(2), 348-365. doi:10.1124/pr.109.001933

Strauss, L., Brink, C. B., Moller, M., Stein, D. J., & Harvey, B. H. (2014). Late-Life Effects of Chronic Methamphetamine Exposure During Puberty on Behaviour and Corticostriatal Mono-Amines in Social Isolation-Reared Rats. *Developmental Neuroscience, 36*(1), 18-28. doi:10.1159/000357495

Struntz, K. H., & Siegel, J. A. (2018). Effects of Methamphetamine Exposure on Anxiety-Like Behavior in the Open Field Test, Corticosterone, and Hippocampal Tyrosine Hydroxylase in Adolescent and Adult Mice. *Behavioural Brain Research, 348*, 211-218. doi:10.1016/j.bbr.2018.04.019

Swant, J., Chirwa, S., Stanwood, G., & Khoshbouei, H. (2010). Methamphetamine Reduces Ltp and Increases Baseline Synaptic Transmission in the Ca1 Region of Mouse Hippocampus. *PLoS One, 5*(6), e11382. doi:10.1371/journal.pone.0011382

Taylor, S. B., Lewis, C. R., & Olive, M. F. (2013). The Neurocircuitry of Illicit Psychostimulant Addiction: Acute and Chronic Effects in Humans. *Substance Abuse and Rehabilitation, 4,* 29-43. doi:10.2147/sar.s39684

Teixeira-Gomes, A., Costa, V. M., Feio-Azevedo, R., Bastos, M. d. L., Carvalho, F., & Capela, J. P. (2015). The Neurotoxicity of Amphetamines During the Adolescent Period. *International Journal of Developmental Neuroscience, 41*(1), 44-62. doi:10.1016/j.ijdevneu.2014.12.001

Tu, G., Ying, L., Ye, L., Zhao, J., Liu, N., Li, J., . . . Zhang, L. (2019). Dopamine D1 and D2 Receptors Differentially Regulate Rac1 and Cdc42 Signaling in the Nucleus Accumbens to Modulate Behavioral and Structural Plasticity after Repeated Methamphetamine Treatment. *Biological Psychiatry, 86*(11), 820-835. doi:10.1016/j.biopsych.2019.03.966

Tucker, L. B., & McCabe, J. T. (2017). Behavior of Male and Female C57bl/6j Mice Is More Consistent with Repeated Trials in the Elevated Zero Maze Than in the Elevated Plus Maze. *Frontiers in Behavioral Neuroscience, 11*, 13. doi:10.3389/fnbeh.2017.00013

Volkow, N. D., & Morales, M. (2015). The Brain on Drugs: From Reward to Addiction. *Cell, 162*(4), 712-725. doi:10.1016/j.cell.2015.07.046

Vorhees, C. V., Reed, T. M., Morford, L. L., Fukumura, M., Wood, S. L., Brown, C. A., . . . Williams, M. T. (2005). Periadolescent Rats (P41-50) Exhibit Increased Susceptibility to D-Methamphetamine-Induced Long-Term Spatial and Sequential Learning Deficits Compared to Juvenile (P21-30 or P31-40) or Adult Rats (P51-60). *Neurotoxicology and Teratology, 27*(1), 117-134. doi:10.1016/j.ntt.2004.09.005

Wahlstrom, D., Collins, P., White, T., & Luciana, M. (2010). Developmental Changes in Dopamine Neurotransmission in Adolescence: Behavioral Implications and Issues in Assessment. *Brain and Cognition, 72*(1), 146-159. doi:10.1016/j.bandc.2009.10.013

Wall, P. M., & Messier, C. (2000). Concurrent Modulation of Anxiety and Memory. *Behavioural Brain Research, 109*(2), 229-241. doi:10.1016/s0166-4328(99)00177-1

Wall, P. M., & Messier, C. (2002). Infralimbic Kappa Opioid and Muscarinic M1 Receptor Interactions in the Concurrent Modulation of Anxiety and Memory. *Psychopharmacology, 160*(3), 233-244. doi:10.1007/s00213-001-0979-9

White, I. M., Minamoto, T., Odell, J. R., Mayhorn, J., & White, W. (2009). Brief Exposure to Methamphetamine (Meth) and Phencyclidine (Pcp) During Late Development Leads to Long-Term Learning Deficits in Rats. *Brain Research, 1266*, 72-86. doi:10.1016/j.brainres.2009.02.024

Wilhelm, C. J., Johnson, R. A., Eshleman, A. J., & Janowsky, A. (2008). Lobeline Effects on Tonic and Methamphetamine-Induced Dopamine Release. *Biochemical Pharmacology, 75*(6), 1411-1415. doi:10.1016/j.bcp.2007.11.019

Williams, G. V., & Castner, S. A. (2006). Under the Curve: Critical Issues for Elucidating D1 Receptor Function in Working Memory. *Neuroscience, 139*(1), 263-276. doi:10.1016/j.neuroscience.2005.09.028

Wiskerke, J., Schetters, D., van Es, I. E., van Mourik, Y., den Hollander, B. R., Schoffelmeer, A. N., & Pattij, T. (2011). Mu-Opioid Receptors in the Nucleus Accumbens Shell Region Mediate the Effects of Amphetamine on Inhibitory Control but Not Impulsive Choice. *The Journal of Neuroscience, 31*(1), 262-272. doi:10.1523/jneurosci.4794-10.2011

Yan, P., Xu, D., Ji, Y., Yin, F., Cui, J., Su, R., . . . Lai, J. (2019). Licl Pretreatment Ameliorates Adolescent Methamphetamine Exposure-Induced Long-Term Alterations in Behavior and Hippocampal Ultrastructure in Adulthood in Mice. *International Journal of Neuropsychopharmacology, 22*(4), 303-316. doi:10.1093/ijnp/pyz001

Ye, T., Pozos, H., Phillips, T. J., & Izquierdo, A. (2014). Long-Term Effects of Exposure to Methamphetamine in Adolescent Rats. *Drug and Alcohol Dependence, 138*, 17-23. doi:10.1016/j.drugalcdep.2014.02.021

Zahrt, J., Taylor, J. R., Mathew, R. G., & Arnsten, A. F. (1997). Supranormal Stimulation of D1 Dopamine Receptors in the Rodent Prefrontal Cortex Impairs Spatial Working Memory Performance. *The Journal of Neuroscience, 17*(21), 8528-8535.

Zakharova, E., Leoni, G., Kichko, I., & Izenwasser, S. (2009). Differential Effects of Methamphetamine and Cocaine on Conditioned Place Preference and Locomotor Activity in Adult and Adolescent Male Rats. *Behavioural Brain Research, 198*(1), 45-50. doi:10.1016/j.bbr.2008.10.019

Zarrindast, M. R., Babapoor-Farrokhran, S., Babapoor-Farrokhran, S., & Rezayof, A. (2008). Involvement of Opioidergic System of the Ventral Hippocampus, the Nucleus Accumbens or the Central Amygdala in Anxiety-Related Behavior. *Life Sciences, 82*(23-24), 1175-1181. doi:10.1016/j.lfs.2008.03.020

Zarrindast, M. R., & Khakpai, F. (2015). The Modulatory Role of Dopamine in Anxiety-Like Behavior. *Archives of Iranian Medicine, 18*(9), 591-603. doi:0151809/aim.009

Zombeck, J. A., Gupta, T., & Rhodes, J. S. (2009). Evaluation of a Pharmacokinetic Hypothesis for Reduced Locomotor Stimulation from Methamphetamine and Cocaine in Adolescent Versus Adult Male C57bl/6j Mice. *Psychopharmacology, 201*(4), 589-599. doi:10.1007/s00213-008-1327-0